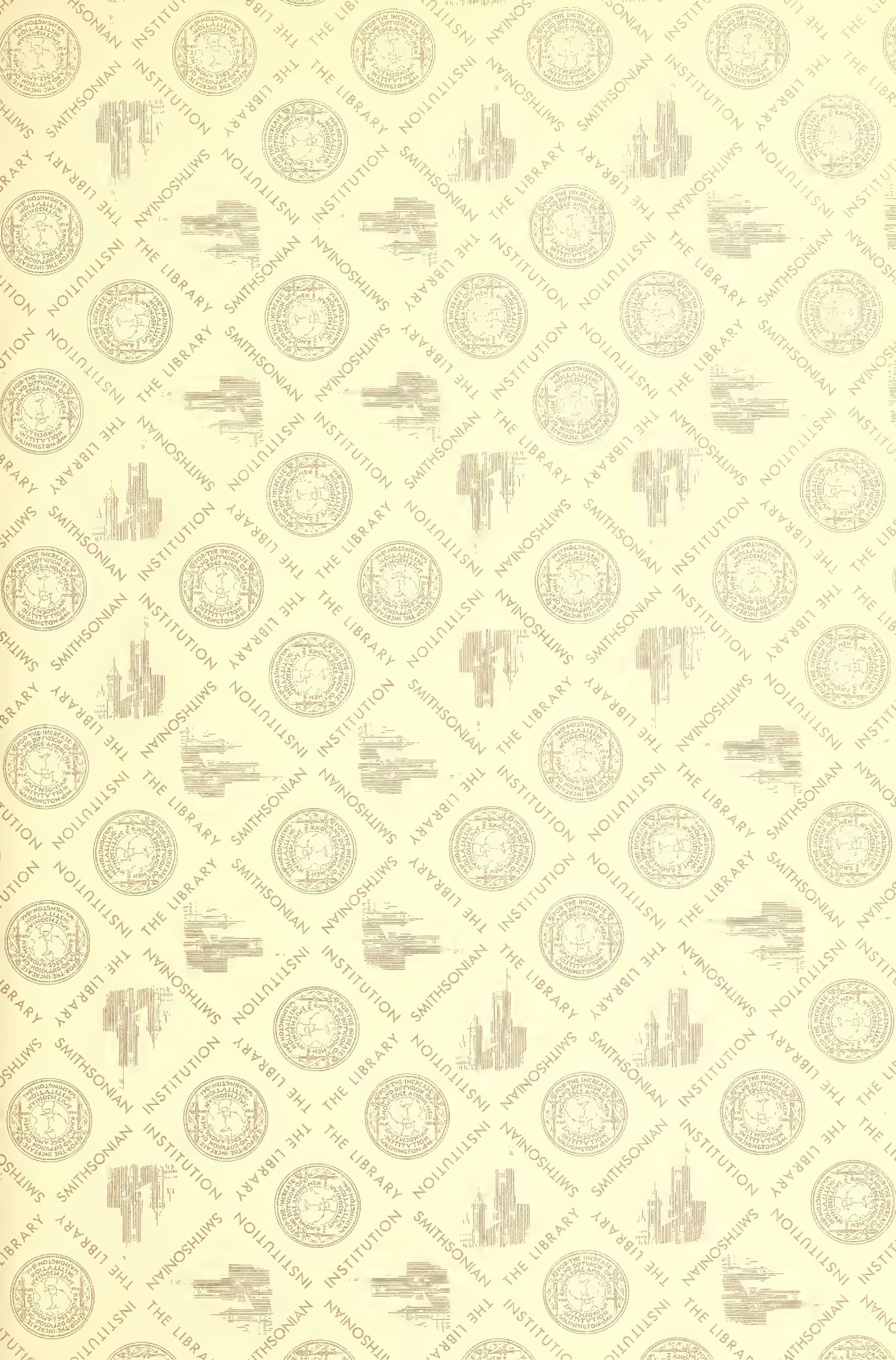
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Issue 1. April 28, 1964

Issue 2. July 7, 1964

Issue 3. November 2, 1964

Issue 4. January 15, 1965

~~Issue 4. December 31, 1964~~

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Published April 28, 1964

1

Breeding Seasons and Annual Cycles of Trinidad Land-birds[1]

D. W. Snow & B. K. Snow
Department of Tropical Research, New York Zoological Society

(Text-figures 1-11)

[This paper is a contribution from the William Beebe Tropical Research Station of the New York Zoological Society at Simla, Arima Valley, Trinidad, West Indies. The Station was founded in 1950 by the Zoological Society's Department of Tropical Research, under Dr. Beebe's direction. It comprises 200 acres in the middle of the Northern Range, which includes large stretches of government forest reserves. The altitude of the research area is 500 to 1,800 feet, with an annual rainfall of more than 100 inches.

For further ecological details of meteorology and biotic zones see "Introduction to the Ecology of the Arima Valley, Trinidad, B.W.I.," by William Beebe, Zoologica, 1952, Vol. 37, No. 13, pp. 157-184].

INTRODUCTION

A SURVEY of the breeding seasons of the land-birds was one of the principal investigations undertaken during 4½ years' residence at the New York Zoological Society's field station, now the William Beebe Tropical Research Station, situated in the Arima Valley in the center of the Northern Range of Trinidad (Text-fig. 1). This paper presents the results of the survey and attempts to relate the Trinidad breeding seasons to the general pattern of breeding in the northern part of South America.

The basic field work consisted of the finding and recording of nests and the systematic trapping and examining of as great a variety of species as possible in every month of the year. Those species that nest in traditional sites, or whose nests are abundant and easily found, could be investigated systematically and their breeding seasons accurately determined, but the nests of others are difficult to find and some were never found. Likewise, some species were trapped in large numbers, others in small numbers irregularly, and others not at all. Thus the information on the various species is necessarily of varying degrees of completeness. The biggest gap is the absence of any records for the diurnal birds of prey, which in some other parts of the tropics have been found to have somewhat different breeding seasons from most other land-birds (*e.g.*, Africa; Moreau, 1950).

A special effort was made to determine the breeding seasons of several forest species accurately, by regular searching along certain stretches of forest stream and trail in the Arima Valley. These "beats" were covered approximately weekly throughout the year, some for 4½ years and others for shorter periods. As a result, the breeding seasons of five species whose nests could be found in large numbers were determined with certainty, and even for species of which only 10 or 20 nests were found by this means the information so obtained is probably reliable.

For three years, a systematic weekly search was also made in a few acres of orchard at Arima, four miles from the main study area. Here, where there were only well-spaced small trees and bushes which could easily be inspected, a fairly complete account could be kept of the nesting of a limited number of typically savanna species.

As mentioned in the next section, a large number of nest records also came from the Sangre Grande area in eastern Trinidad, mainly from cultivated country with citrus, cocoa and small gardens. This area, some 15 miles east of the Arima Valley, has a less seasonal climate with a very high rainfall. Smaller numbers of records came from savanna country along the southern edge of the Northern Range, from the

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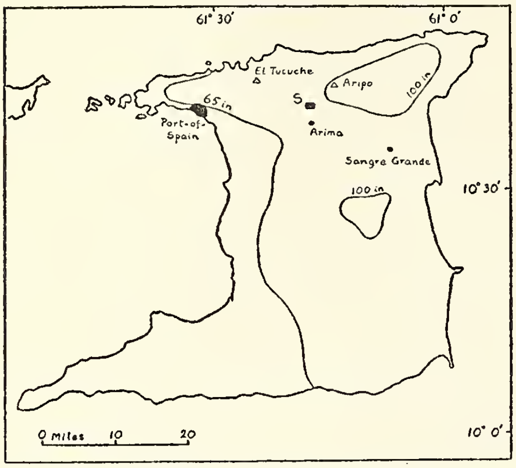
[1] Contribution No. 1,052, Department of Tropical Research, New York Zoological Society.



TEXT-FIG. 1. Map of Trinidad, showing 100-inch and 65-inch isohyets. **S**: the main study area, in the central part of the Arima Valley.

Port-of-Spain area in the northwest of the island, where the climate is drier than in the Arima Valley, and from various other localities, but none from the extreme south of the island. Thus there are records from forest, savanna, plantations and gardens, but the different areas of Trinidad are unequally represented.

In addition to the actual finding of nests, a small number of breeding records were based on the observation of recently fledged young birds. But except for a few specimens with full-sized eggs in the oviduct, gonad state was not used, although a considerable number of specimens was examined. For some species at least, fully developed testes in the male are not a good guide to the breeding season, since a state of full development is maintained for far longer than the female's egg-laying period.

The trapping and banding program provided important ancillary information on the season of moult. Indeed, as will become clear, in a study which is essentially concerned not only with the breeding season but with the whole annual cycle, knowledge of the season of moult is perhaps even more important than knowledge of the breeding season, as it seems to be the less variable of the two and may be more directly connected with the timing of the annual cycle.

Systematic recording of song-periods gave further information on the annual cycle. Weekly notes were made on the songs of 39 species, most of them for a period of three years or more. For many of them, the song period was found to coincide closely with the breeding season (as Skutch (1950) has also found for Central America), so that for those whose breeding season is not well known the evidence from the song period may be a useful guide as to its probable extent.

ACKNOWLEDGMENTS

By great good fortune, shortly before this investigation began, the Trinidad Regional Virus Laboratory began a virological study of nestling birds which continued for over three years, from 1956 to 1959. In the course of this work one or two field assistants were employed throughout the year in systematic searching for nests in an area some 15 miles east of the Arima Valley. From April, 1957, one of us was associated with this work and an effort was made to improve and standardize the field assistants' recording methods. We are extremely grateful to Dr. W. G. Downs, former Director of the Trinidad Regional Virus Laboratory, for putting these records, amounting to over 900, at our disposal, and to him and Dr. T. H. G. Aitken for their help in the field in the course of this program.

Early in 1957, an attempt was made at a meeting of the Trinidad Field Naturalists' Club to introduce a nest record scheme for Trinidad, like those now operating successfully and on an increasing scale in several northern countries. The response was poor, and it became clear that there is as yet too small a number of people deeply enough interested in natural history for such a scheme to work, but three keen amateur ornithologists, Commander C. S. Bushe, Mr. R. P. ffrench and Mr. J. Dunston, maintained a continuous and valuable supply of nest records, and Dr. V. C. Quesnel supplied some old records. The record cards thus received totalled over 200 and formed a valuable addition to the collected data for which we are most grateful. We are also grateful for smaller numbers of unpublished records from Dr. William Beebe, Mr. W. Conway and Mr. C. T. Collins.

R. P. ffrench and C. T. Collins, working in the same areas as we worked and using the same methods, continued to trap and record moult in the year after our departure from Trinidad. Their data, which they have generously put at our disposal, add substantially to our own records.

Finally, we acknowledge with gratitude National Science Foundation Grants G 4385 and G 21007, without which this study could not have been carried out.

THE PUBLISHED RECORDS

Williams (1922) gave precise and detailed records of the breeding of a limited number of species in Trinidad, based on residence over several years; these have been incorporated with our own data. Belcher & Smooker's series of papers (1934-37) provide a great deal of information on the breeding of Trinidad birds, which has recently been incorporated in a standard work (Herklots, 1961). Yet the information given in these papers is unsatisfactory in several ways. In the first place, for many species we have found that the breeding seasons given by Belcher & Smooker are far too short. Secondly, for most species they give a general statement, without specifying the number of records on which it is based. Thirdly, some of the nests were undoubtedly wrongly identified. Consequently, we have used their information only in cases where they clearly record all the nests found and there is no question of misidentification.

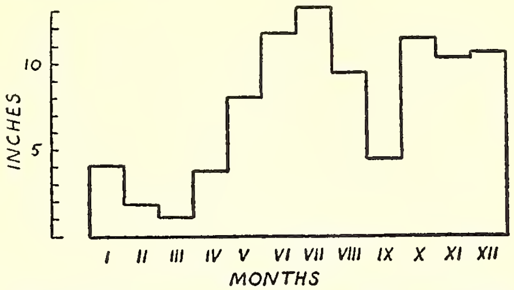
There have been a few other published records. Most concern species of which we have adequate records, and have not been used; but a small number refer to species whose breeding is little known (*e.g., Nyctibius griseus, Ramphastos vitellinus, Formicarius analis*), and these have been used. The source of all the records is given in the tabulated summary of breeding forming the Appendix to this paper.

The Environment

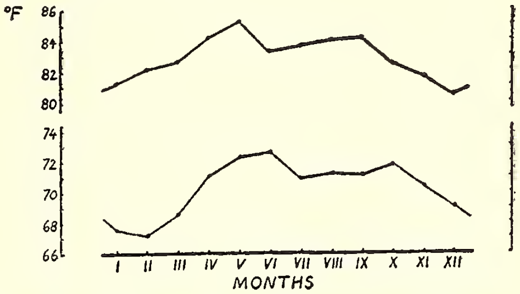
Lying between latitudes 10° and 11° north, Trinidad has a generally humid climate and supports a natural vegetation which is almost entirely forest, ranging from rain-forest in the wetter parts of the lowlands and hills, through semi-seasonal and seasonal forests over the greater part of the lowlands, to strongly seasonal monsoon forest in the driest areas in the northwest (Beard, 1946).

Physical Factors

As in most parts of the tropics, the seasons are determined mainly by the changing rainfall. There is a single long dry season, beginning in January and ending variably, usually in May, and a single wet season lasting for the rest of the year, which is often broken by a short dry spell (the "petit carême") in September or October (Text-fig. 2). During the dry season there is usually some sporadic rainfall, which may be heavy, in each month; precipitation averages an inch or two in February and March, the two driest months. In the wet season monthly averages of over 10 inches are common, except in the drier western parts of the island and on the coasts.



Text-fig. 2. Means monthly rainfall, Arima Valley, 1957-1959. (Text-fig. 10 shows the rainfall for the five years 1934-1938.)



Text-fig. 3. Mean monthly maximum and minimum temperatures, St. Patrick's Estate, Arima Valley.

Mean temperature varies rather little, but is slightly lower from November to March than in the other months, and this is due especially to a decrease in the nightly minima. To this extent the "winter" is still felt only ten degrees from the Equator. The rise in mean minima from February to May constitutes the most striking temperature change of the year (Text-fig.3).

The trade winds blow persistently from the northeast during the dry season, and the weather then is at its pleasantest, especially in January and February when night temperatures regularly fall to the lower 60's F. (16-18°C.) In the wet season the wind tends to blow from the southeast and is more variable; nights are warmer and more humid, and day temperatures more variable than in the dry season because of the varying cloud cover. The rain usually falls in heavy downpours and wet spells rarely last for more than two days. At all seasons of the year there is much sunshine.

At 10° 40′ N., the latitude of the Arima Valley where most of the observations were made, day-length varies by only 75 minutes in the course of the year, the longest day being 37½ minutes more and the shortest 37½ minutes less than 12 hours. The fastest rate of change of day-length, at the time of the equinoxes, is a little under half a minute per day, while for a considerable period at the summer and winter solstices day-length is practically constant.

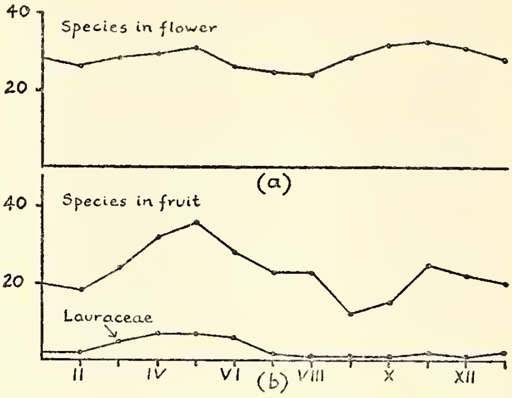
The Vegetation

As already mentioned, the wettest parts of Trinidad are in the northeast. Here the natural vegetation is evergreen forest. The driest part is the northwest corner. Here, at the extreme northwest point of Trinidad, and especially on the off-lying islands which reach out towards the Paria peninsula of Venezuela, a xerophytic and mainly deciduous vegetation prevails, in which cactuses are prominent. A distance of only 30 miles separates these extremes. The natural vegetation of the greater part of Trinidad, with annual rainfalls of 70-100 inches, is lowland forest which according to rainfall is more or less seasonal, *i.e.*, contains a more or less important deciduous element. There is also some natural savanna, edaphic in origin, lying mainly in a belt along the southern edge of the Northern Range, and some mangrove swamp and other forms of coastal vegetation.

It will be apparent that conditions in the Arima Valley, almost exactly in the middle of the Northern Range, must be intermediate between the wet and the dry extremes. In fact its climate is nearer the wet extreme, the annual rainfall averaging nearly 100 inches, and the natural vegetation of the lower part of the valley, the center of the study area, is transitional between lowland seasonal forest and lower montane rain forest, as defined by Beard (1946). Further details of the climate and vegetation of the Arima Valley are given in Beebe (1952) and Snow (1962a).

Especially in the western part of the island, much of the original forest has now been cleared, swamps have been drained and there is extensive cultivation of sugar, citrus fruits, cocoa, coconut and other tropical crops. But nearly 30% of the land surface of Trinidad is still under forest.

The changing seasons affect the appearance of the forest strikingly. Most of the trees have well-defined flowering and fruiting seasons, many have well-marked seasons of leaf fall and renewal of leaf, and all the individual trees of the same species are usually well synchronized in any given area. In general, there is most loss of leaf during the dry season, and most renewal of leaf in the wet season. Trees with conspicuous blossoms flower mainly at the end of the wet season and in the dry season (December-May), so that one's first impression is that this is the main flowering season. But many species are in flower in every month, and more systematic observation showed that in our area the number of tree species in flower was somewhat greater in May and October-December than in other months (Text-fig. 4a). For shrubs and herbs, the seasonal variation in flowering was rather similar, but many of them have much more protracted flowering seasons than the trees. However, it is important to note that many of the trees and shrubs that flower in the wet months have small, relatively inconspicuous flowers, and though these are exploited by the specialized nectar-eating birds, the supply of nectar is probably greatest in the dry season.



TEXT-FIG. 4. Flowering and fruiting seasons in the Arima Valley. (a) number of tree species in flower in different months; (b) number of tree species in fruit; upper line, all species; lower line, Lauraceae.

Ripe fruits of many kinds are available throughout the year, but more different kinds ripen in the months April-June (the end of the dry and beginning of the wet season) than at any other time. There is also a second, minor peak of fruit abundance in November (Text-fig. 4b).

The rather ill-defined peaks of flowering (in May and November) coincide with the two much better defined but more unequal peaks of fruiting, and appear to some extent to be related to them. Thus many of the trees that contribute to the November peak of flowering ripen their fruit in the following dry season and so contribute to the April-June peak of fruiting, and similarly many of those that contribute to the May peak of flowering have ripe fruit at the end of the year and so contribute to the November peak of fruiting. But we made systematic observations on the fruiting seasons only of trees with fleshy fruits, eaten by birds, and the above generalizations may not apply, and indeed in individual cases do not apply, to trees producing other kinds of fruit.

Insect life is abundant at all times, but is probably at its height at the beginning of the wet season. It is then that mosquitoes and Lepidoptera breed most actively and termites swarm after heavy falls of rain. Then, too, the invertebrates of the forest floor become more accessible to ground-feeding birds as the earth, parched after weeks of dry weather, is again softened. Thus both for insectivorous and frugivorous birds food is probably at its most abundant in the early part of the wet season.

Breeding Seasons of the Land-birds

Presentation of the Results

As in most recent studies, the breeding date is taken to be the date on which the first egg was known or calculated to have been laid. For convenience, the data are grouped by months, in spite of the fact that all months are not of quite the same length. In the small proportion of cases where the calculated laying date falls within a period spanning two months, the middle date has been used.

The breeding and moult records for all species are tabulated in the Appendix, which is designed to be used as a general reference to the sections that follow. Fuller details are given in these sections of the breeding and moulting seasons of those species for which the information is especially complete.

Unless otherwise stated, "moult" and "moulting" refer to the complete moult, involving the flight- and tail-feathers as well as the body-feathers and coverts. In the majority of the birds dealt with here, as in north-temperate birds, there is an annual complete moult following immediately after the breeding season. Partial moults, involving only the body-feathers and wing- and tail-coverts, are in general more protracted and less regularly seasonal. In many of the species which we trapped in good numbers, it appeared to be the rule for juveniles to undergo a partial moult in the course of their first few months after leaving the nest, and for their next moult, which was complete, to be more or less synchronous with the adults' post-breeding moult.

The replacement of the flight-feathers spans practically the entire period required for the replacement of the whole plumage, and so is a convenient criterion for identifying a bird undergoing a complete moult. There are further reasons why it is the only safe criterion to use when examining unknown individuals under field conditions. Such replacement of the body-plumage as may continue for a time after the wing-moult is complete cannot, in many species, be distinguished from partial moult; and a certain number of body-feathers, and also tail-feathers, may be found growing at any time, presumably due to accidental loss.

Tinamou (Tinamidae)

The nine records for the single species, *Crypturellus soui,* are from nine different months. We have no record for May, undoubtedly a chance gap as Belcher & Smooker say that most eggs are laid in that month. Calling is heard throughout the year, but we do not know whether it is associated with breeding activities. The evidence suggests a more or less continuous breeding season, with no very marked peaks. We have no moult records.

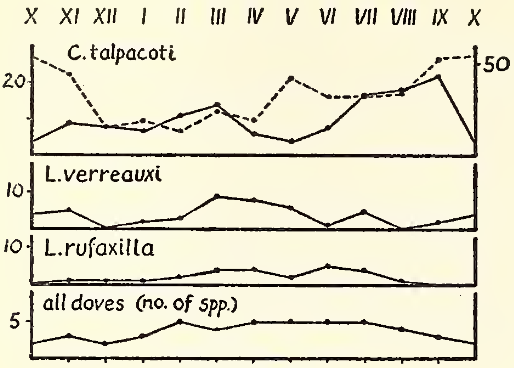
Pigeons and Doves (Columbidae)

A large number of records were obtained for *Columbigallina talpacoti,* and good numbers for *Leptotila rufaxilla* and *L. verreauxi.* All have very long breeding seasons (Tables I and II; Text-fig. 5a).

C. talpacoti was one of the few species found breeding in every month of the year. In an area of a few acres of orchard and garden at Arima which was searched systematically for three years, 60 nests were found, and 53 were found by less systematic searching by Trinidad Regional Virus Laboratory field assistants over 3½ years, near Sangre Grande 15 miles east of Arima (Table I). The Arima nests were grouped in two main periods, with greatest numbers in July-September and November-January and an almost complete gap in March-May (two nests only); and the pattern was similar in each year. The seasonal distribution of the Sangre Grande nests was rather different: they were recorded in every month except December, and 21% of them were in the months March-May.

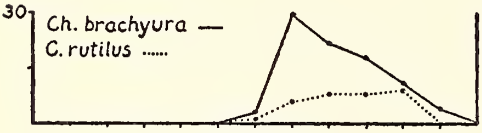
The difference between the two sets of records may have been partly the result of different local climates. At Arima, with a fairly severe dry season, the nests were concentrated towards the ends of the two wettest periods of the year, perhaps in response to the ripening of the grass and weed seeds which probably form this dove's main food. At Sangre Grande, situated in the wettest part of the island, the climate may allow a more continuous production of suitable seeds. That the birds were indeed responding to favorable local conditions was suggested by the observations at Arima. Often, three or four nests would be started in a small area within a week or two of each other, after a period of inactivity.

The opportunist nature of the breeding of this species was also indicated by its moult. In the first place, unlike most other species which were examined, birds undergoing wing-moult were caught in nearly every month of the year (Table I). More notably, several of those that were not moulting showed evidence of "arrested" moult (Miller, 1961); that is, they had primary feathers of different ages in the wing, some of the outer ones being plainly older and more worn than the inner ones. In all of them, the moult had been arrested at a late stage, with two, three or four outer primaries still unshed. The most

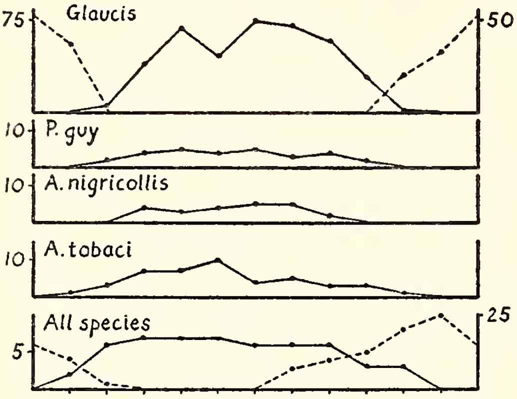


(a) DOVES

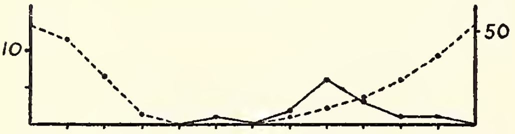
(b) OILBIRD



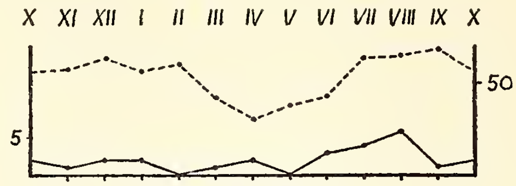
(c) SWIFTS



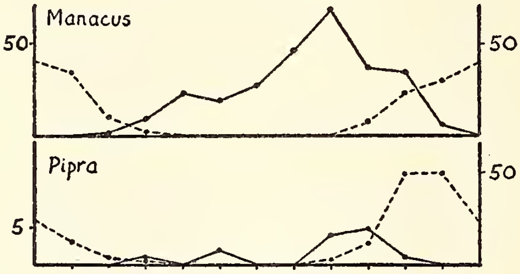
(d) HUMMINGBIRDS



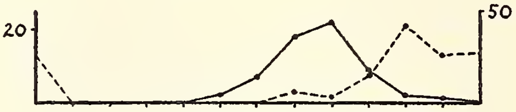
(e) WOODHEWERS



(f) THAMNOPHILUS

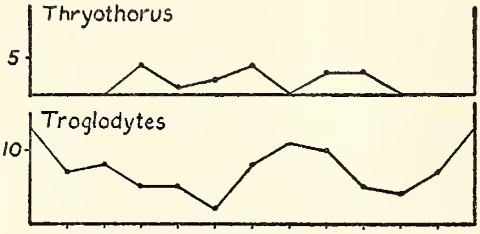


(g) MANAKINS

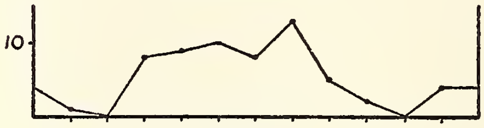


(h) PIPROMORPHA

(i) SWALLOWS

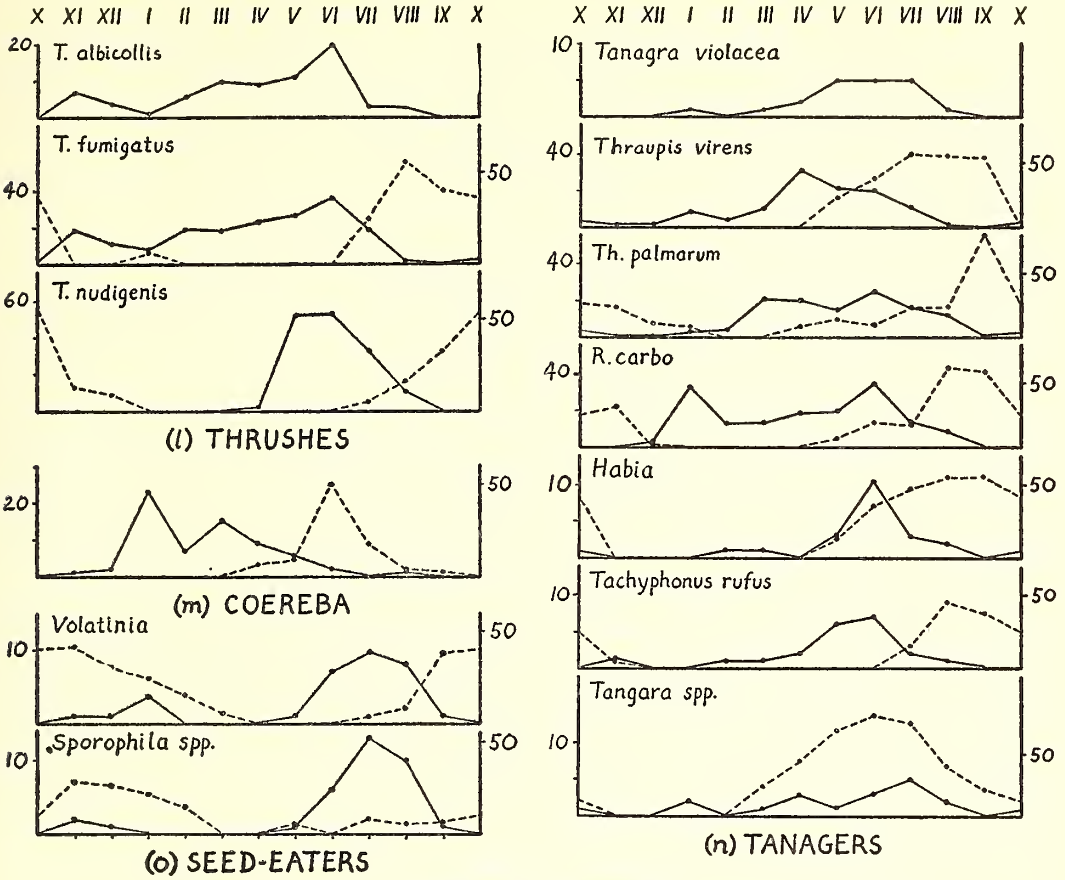


(j) WRENS



(k) MIMUS

TEXT-FIG. 5. Breeding and moulting seasons of different families and species. The number of nests is shown by a solid line, the scale being on the left-hand side, and the percentage of trapped birds in moult by a broken line, the scale being on the right-hand side. (The moulting percentage has been smoothed for *T. nudigenis, Volatinia, Thraupis virens, T. palmarum, Habia* and *Tachyphonus rufus.*)



TEXT-FIG. 5 *(Continued)*

reasonable explanation of this situation is that the birds had begun to moult and then stopped before the wing-moult was complete, presumably because local conditions had stimulated them to breed. Arrested moult has been described in other tropical species, *e.g.*, *Pycnonotus xanthopygos* (Moreau *et al.*, 1947) and *Zonotrichia capensis* (Miller, 1961), and in the latter species it was proved to be connected with a resumption of breeding activities.

It is doubtful whether the figures justify drawing any distinction between the breeding season of *Leptotila verreauxi,* a dove of orchard and plantation, and *L. rufaxilla,* a forest dove. In both the period of greatest activity is March-July. These are also the months when the greatest number of different species of doves were found breeding (Table II).

Parrots (Psittacidae)

The four records for *Touit batavica* are all in the first three months of the year. We have no records for other species, but for *Forpus passerinus* Belcher & Smooker record egg-laying in April and May; and for *Amazona amazonica* the literature records are for March, April and May, and the local people agree that April is the month for collecting nestlings. For the family as a whole, January-May can thus be taken as the main laying period. We have no moult records.

Cuckoos (Cuculidae)

Adequate information was obtained only for *Crotophaga ani.* It was found breeding in every month, with no clear indication of any peak. In Cuba, *C. ani* breeds only in the wet season (Davis, 1940), and in Central America the very similar *C. sulcirostris* also breeds in the wet season (Skutch, 1959). The Trinidad nests, which were mainly found in the wetter eastern half of the island, do indeed show a slight tendency to be concentrated in the wet part of the year: 45 (68%) of them were in the six months following the onset of the rains (May-October); but there was only one in December, one of the wettest months, and eight in February, one of the driest months.

Little information was obtained on the moult.

Table I. Breeding and Moulting of *Columbigallina talpacoti*

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Breeding season at Arima	4	3	1	1	—	3	9	11	15	1	4	8
Breeding season in Sangre Grande area	2	6	12	4	3	4	4	5	6	3	4	—
Number in moult in each month, as a fraction of total trapped	$\frac{2}{44}$	$\frac{10}{38}$	$\frac{0}{2}$	$\frac{0}{0}$	$\frac{1}{2}$	$\frac{2}{5}$	$\frac{1}{5}$	$\frac{3}{8}$	$\frac{1}{1}$	$\frac{2}{2}$	$\frac{2}{6}$	$\frac{7}{17}$

Notes, applying also to Tables II-XIII. The breeding season is shown by the number of nests in which the first egg was laid in the month indicated. The moulting season is shown by the number of individuals trapped which were undergoing wing-moult in the month indicated, as a fraction of the total number trapped in that month.

Nine of the 17 adults examined were moulting their flight-feathers in the months March, June, July, August and November. The sequence of replacement of the wing-feathers is highly irregular, as in some other tropical cuckoos (Stresemann & Stresemann, 1961). Here again there is evidence of emancipation from a fixed annual cycle.

Owls (Strigidae and Tytonidae)

All the nine records for *Otus choliba,* and five of the six for *Glaucidium brasilianum,* are in the months February-May. The literature records for the three other species are also nearly all in the same months: *Ciccaba virgata,* April (2) (Belcher & Smooker); *Pulsatrix perspicillata,* January, March (2) (Cherrie, 1908; Belcher & Smooker); *Tyto alba,* February (2), April, May (Belcher & Smooker). We have no moult records.

Oilbird (Steatornithidae)

The breeding season of *Steatornis* has been dealt with fully in an earlier paper (Snow, 1962 (1963) d). Clutches were laid in every month except October and November, most being from December to May (Text-fig. 5b). Most young are in the nest from April to June, and it was found that this is also the period when the greatest number of the Oilbird's food trees have ripe fruit (Text-fig. 4b). The relationship between breeding and moulting was not clear. Both the breeding cycle, from egg-laying to the fledging of the young, and the moult are very long processes, lasting several months. Most moulting takes place in the months June-November, when there is least egg-laying, but the year is not long enough to accommodate separately the time taken in breeding activities and that needed for the moult, and it seems that the processes of breeding and moulting must frequently overlap.

Nightjars (Caprimulgidae)

Uncertainty of identification makes some of the literature records doubtful, but the bulk of all records are in the months March-May, the full extent being from January to August. We have no moult records.

Potoo (Nyctibiidae)

Five of the six records are in March and April, and the sixth in July. We have no moult records.

Swifts (Apodidae)

The swifts have been dealt with fully in an earlier paper (Snow, 1962b). The two species for which there are adequate records *(Chaetura brachyura* and *Cypseloides rutilus)* breed in the early part of the wet season, from late April or May to late August or September (Text-fig. 5c). Thus their young are in the nest at the time when flying insects are probably at their most abundant. The moult follows close after breeding, birds undergoing wing-moult being nearly all caught in the months August-November.

The suggested adaptive relationship between the swifts' breeding season and the abundance of their food supply is supported by the more limited data for the swallows (p. 12), which exploit a similar food supply–small flying in-

Table II. Breeding Seasons of *Leptotila rufaxilla* and *L. verreauxi*

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Leptotila rufaxilla	1	1	4	4	2	5	3	—	—	—	1	1
Leptotila verreauxi	2	3	9	8	6	1	5	—	2	4	4	—
Number of dove species breeding in each month	3	4	4	5	4	5	5	2	3	2	3	2

sects—and have rather similar breeding seasons. The earlier breeding seasons of the nightjars and potoo, which also take flying insects, may be related to their more exposed nest-sites, favoring breeding in the dry season, or it may be that the larger crepuscular and nocturnal insects on which they feed are more active or more easily visible at this season.

Hummingbirds (Trochilidae)

There is information for eight species, including very full records for *Glaucis hirsuta* and less numerous but adequate records for *Phaethornis guy, Anthracothorax nigricollis, Amazilia tobaci* and *Chrysolampis mosquitus.* The season is a long one, beginning at the end of the year (November in two species, December in three species) and continuing to July or August. All the species for which the data are adequate show approximately the same extent of breeding. The moult takes place mainly from July to November, but a little from May onwards (Text-fig. 5d, Table III).

There was some evidence that breeding is a little later in forest than open country. Thus, in four years, *Glaucis* began in late December and January in open places, and a month or more later in closed forest. The nests of *Amazilia tobaci* in forest in the Arima Valley were all in the months February-July, those in open places from December onwards. The most strictly forest species of all, *Chlorestes notatus,* seemed to be especially late. All our records were in May and June, though Belcher & Smooker give a doubtful record of a March nest and we have seen birds collecting nest-material in late December and February.

In the four species for which records were kept over a period of four years, regular song was recorded throughout the year except for the time of moult, when it was either much reduced *(Amazilia tobaci)* or ceased entirely for several weeks *(Amazilia chionopectus, Phaethornis guy, P. longuemareus).*

The hummingbird breeding season covers the whole of the dry season, with a little breeding starting well before, when the weather is still very wet, and a good deal of breeding continuing for about two months after the wet season has begun. Thus there is no very exact correlation between hummingbird breeding seasons and weather. It has already been mentioned that the number of species of trees and other plants in flower is high in all months of the year, but that in general the trees with large and conspicuous blossoms, which are especially attractive to hummingbirds, flower mainly from December to May, while those that flower in the wet season mainly have smaller flowers which probably provide less nectar. Similarly the most important of the herbs and vines which are exploited by hummingbirds flower mainly in the dry season (especially *Heliconia* spp., *Justicia, Norantea, Pachystachys).* Thus the main part of their breeding season in Trinidad probably coincides with the period of greatest availability of nectar, but here again, due to the length of the breeding season, synchronization cannot be exact. It is significant that, among the other birds for which there is information, the breeding season of *Coereba flaveola,* the most pronounced nectar-feeder, is most like that of the hummingbirds (p. 14).

Kingfishers (Alcedinidae)

We had no breeding records; those given by Belcher & Smooker for three species of *Chloroceryle* are all in the months March-September. Two individuals of *C. americana* were trapped twice. One was moulting in September (moult well advanced) and not in the following July; the other was moulting in November (moult

TABLE III. ANNUAL CYCLES OF EIGHT SPECIES OF HUMMINGBIRDS

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Breeding season of:												
Glaucis hirsuta	38	69	46	75	71	58	28	1	—	—	—	6
Phaethornis guy	4	5	4	5	3	4	2	—	—	—	1	2
Phaethornis longuemareus	4	5	2	—	—	1	—	—	—	—	—	1
Anthracothorax nigricollis	4	3	4	5	5	2	—	—	—	—	—	—
Chrysolampis mosquitus	5	6	5	4	9	—	—	1	—	—	—	1
Chlorestes notatus	—	—	1	—	2	2	—	—	—	—	—	—
Amazilia chionopectus	1	3	2	1	1	—	—	—	—	—	—	1
Amazilia tobaci	7	7	10	4	5	3	3	1	—	—	1	3
Number of species breeding in each month	7	7	8	6	7	6	3	3	—	—	2	6
Number of species moulting in each month	—	—	—	—	1	1	4	5	6	5	4	1

nearly completed) and not in the following February. Six other individuals trapped in December, April, May and June were not moulting. These records, though few, suggest that August-November are the usual months of moult.

Trogons (Trogonidae)

The six breeding records available are well distributed throughout the year, with the suggestion of some concentration in the months February-May. One bird trapped in July was moulting; six others, trapped in March, June, July and October were not. Calling was recorded throughout the year. The evidence points to a long and perhaps ill-defined breeding season, but is too scanty for any firm conclusion.

Motmot (Momotidae)

All seven breeding records for the single species, *Momotus momota*, are in April and May. The moult records are in June, July and October. The moult must take a long time for each individual, as a bird which was in an early stage of wing-moult on June 26 (8 old primaries remaining) was still moulting on October 1 (2 old primaries remaining).

Jacamar (Galbulidae)

All except one of the 17 records for the single species, *Galbula ruficauda*, are in the months February-April, the exception being in June. We have only a single moult record, also in June.

Toucan (Ramphastidae)

The four records, all from the literature, for the single species, *Ramphastos vitellinus*, are from March to June (Belcher & Smooker; Chenery, 1956). We have no moult records.

Woodpeckers (Picidae)

The best information is for *Celeus elegans* and *Piculus rubiginosus*. Breeding records are in the months April-May and March-May respectively, and moult records in July-November and August-September respectively. In *Celeus* the moult must take well over four months for the individual, as one bird trapped twice while moulting had replaced only four of the ten primaries in nine weeks. *Veniliornis kirkii* appears, from the few records, to breed a little earlier than these two. Belcher & Smooker's records for *Ceophloeus lineatus* and *Phloeceastes melanoleucus* suggest breeding seasons similar to those of *Celeus* and *Piculus*.

Woodhewers (Dendrocolaptidae)

Few breeding records were obtained for the two common species, *Xiphorhynchus guttatus* and *Dendrocincla fuliginosa*, but combined with the moult records and their periods of calling they gave a reasonable picture of the annual cycle (Table IV, Text-fig. 5e). Both species breed mainly in the first half of the wet season (May-July), and moult in the second half of the year. The full extent of the breeding season appears to be from March to September, the records for *Xiphorhynchus* being on average earlier than those for *Dendrocincla*. Both species begin to call soon after the beginning of the year and continue until early October, with *Xiphorhynchus* again starting rather earlier than *Dendrocincla* (usually mid-January and mid-February respectively).

Spinetails (Furnariidae)

The four species for which there are records are ecologically very different from each other. *Sclerurus albigularis*, a ground-living forest bird and a tunnel-nester, has a breeding season which is apparently confined to the "winter," having its peak in December-January, a season unmatched by any other Trinidad bird. *Synallaxis albescens*, a species of semi-open country, has an extended breeding season, the 17 records being from eight different months, and that of *S. cinnamomea*, a forest species, appears also to be extended. *Certhiaxis cinnamomea*, a swamp bird, breeds from June to October, a restricted and late breeding season similar to

TABLE IV. ANNUAL CYCLES OF TWO SPECIES OF WOODHEWERS

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Dendrocincla fuliginosa												
Breeding season	—	—	—	—	2	4	1	1	1	—	—	—
Number in moult in each month, as a fraction of the total trapped	$\frac{0}{7}$	$\frac{0}{6}$	$\frac{0}{7}$	$\frac{0}{6}$	$\frac{0}{4}$	$\frac{1}{12}$	$\frac{0}{14}$	$\frac{2}{9}$	$\frac{2}{4}$	$\frac{0}{1}$	$\frac{4}{6}$	$\frac{1}{2}$
Xiphorhynchus guttatus												
Breeding season	—	—	1	—	—	2	2	—	—	—	—	—
Number in moult in each month, as a fraction of the total trapped	$\frac{0}{2}$	$\frac{0}{1}$	$\frac{0}{2}$	$\frac{0}{2}$	$\frac{0}{0}$	$\frac{1}{2}$	$\frac{1}{1}$	$\frac{1}{3}$	$\frac{2}{2}$	$\frac{0}{0}$	$\frac{0}{2}$	$\frac{0}{0}$

that of other passerine birds confined to swamps (*Fluvicola, Arundinicola, Agelaius;* see later).

Moult data were obtained only for *Certhiaxis,* in which two out of the three birds trapped in both November and February were undergoing wing-moult.

Antbirds (Formicariidae)

Antbird nests are notoriously difficult to find, and disappointingly few records were obtained for this family. Only for *Thamnophilus doliatus* do the records form a satisfactory basis for discussion. This species has an extended breeding season, with nests recorded in ten of the twelve months (Table V, Text-fig. 5f). It was also found to moult in almost every month of the year. The percentage of trapped birds undergoing moult was unusually high, and irregularities in the sequence of the wing-moult were far commoner than is usual in passerine birds. *Thamnophilus* lives in pairs throughout the year, calling is heard in all months, and it is difficult to avoid the conclusion that the species as a whole has no well-defined annual cycle (though individual pairs may show annual periodicity). Observations in Tobago (25 miles northeast of Trinidad) suggested that there may be local outbursts of breeding, stimulated by favorable conditions. Thus in July, 1961, during rather wet weather, four occupied nests were found in a limited area where none had been found on previous visits.

Taraba major, Formicarius analis and *Myrmeciza longipes* were also found to call throughout the year, with no marked fluctuations in intensity, and less complete records suggested the same for *Dysithamnus mentalis.* The few records for species other than *Thamnophilus* suggest that breeding may be at its height in the months May-August, but are quite inadequate for further discussion.

Cotingas (Cotingidae)

Too few breeding records are available for *Pachyrhamphus, Attila* or *Tityra* to permit generalizations. Six breeding records of *Procnias averano* were obtained in the course of a detailed study of this species to be published elsewhere (B. K. Snow, in preparation), and there is one dated literature record (Beebe, 1954). These records, and field observations of display and copulation, indicate that the breeding season is in two parts, with a short laying period in late October and November and a more extended laying period in the months April-August. The moult is at its height in September. The moulting season of the other species is not known, but the otherwise more or less continuous calling of *Attila* is considerably reduced in the months July-September, which suggests that for it, too, this is the time of moult.

Manakins (Pipridae)

The breeding seasons of the two species of manakins have been dealt with in earlier papers (Snow, 1962a and 1962 (1963) c). The data are very full for *Manacus manacus;* for *Pipra erythrocephala* the information is less good, but its breeding season seems to be essentially the same. The full extent of the breeding season is from early January, or exceptionally the end of December, to early September, but breeding activity is variable and not usually intense in the first three months of the year, and it does not reach its peak until April-June. Both species moult at the same season, mainly from August to November (Table VI, Text-fig. 5g). It was shown in the earlier papers that more kinds of fruits suitable for manakins are available in the months April-June than at any other time of year (see also Text-fig. 4b), and as insects are probably also at their most abundant at this time it was concluded that there were strong grounds for postulating an adaptive relationship between the breeding season and the seasonal fluctuations in food supply. In *Manacus,* there were annual differences of several weeks in the time of the start of breeding, the significance of which is discussed later (p. 18).

Flycatchers (Tyrannidae)

The flycatchers are an ecologically very diverse group of predominantly insectivorous birds. The 18 species for which data were obtained can be divided fairly satisfactorily into four groups by habitat: birds of forest, open woodland and plantation, savanna, and swamp. The six forest species have, as a group, a well-defined breeding season from March to September (Table VII). This is also the extent of the

TABLE V. BREEDING AND MOULTING OF *Thamnophilus doliatus*

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Breeding season	3	3	—	1	2	3	2	—	2	1	1	1
Number in moult in each month, as a fraction of the total trapped	$\frac{2}{2}$	$\frac{0}{1}$	$\frac{1}{2}$	$\frac{2}{4}$	$\frac{0}{4}$	$\frac{3}{5}$	$\frac{4}{7}$	$\frac{6}{8}$	$\frac{1}{2}$	$\frac{2}{3}$	$\frac{1}{2}$	$\frac{2}{4}$

Table VI. Annual Cycles of *Manacus manacus* and *Pipra erythrocephala*

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Manacus manacus												
Breeding season	9	23	19	27	46	69	37	35	6	—	—	1
Number in moult in each month, as a fraction of the total trapped	$\frac{1}{46}$	$\frac{0}{23}$	$\frac{0}{22}$	$\frac{0}{38}$	$\frac{0}{49}$	$\frac{1}{111}$	$\frac{6}{76}$	$\frac{23}{88}$	$\frac{18}{60}$	$\frac{13}{32}$	$\frac{18}{56}$	$\frac{5}{47}$
Pipra erythrocephala												
Breeding season	1	—	2	—	—	4	5	1	—	—	—	—
Number in moult in each month, as a fraction of the total trapped	$\frac{0}{74}$	$\frac{0}{34}$	$\frac{1}{46}$	$\frac{0}{30}$	$\frac{0}{36}$	$\frac{1}{72}$	$\frac{8}{64}$	$\frac{52}{104}$	$\frac{46}{92}$	$\frac{14}{58}$	$\frac{10}{84}$	$\frac{3}{78}$

breeding season of the single species for which a large number of records were obtained, *Pipromorpha oleaginea* (Table VIII, Text-fig. 5h). The seven species characteristic of open woodland and plantation have a longer breeding season, December and January being the only months in which no records were obtained, with a poorly marked peak in March-July; while the four savanna species were found breeding throughout the year, except for September. The ecological distinctiveness of the species placed in these two latter groups is less clear than between the other groups, and the habitats themselves intergrade.

There are only two species in the last group, and they appear to have a well-defined breeding season which is later than that of the forest group, being at its height from June to October. In this they agree with the other land-birds inhabiting swamps, and there is an obvious adaptive basis for their late breeding season. The swamps largely dry out during the dry season and flood again in the course of the wet season. The main annual increase in the invertebrate fauna probably coincides with the first months of rising water-level.

Swallows (Hirundinidae)

The 14 breeding records for *Progne chalybea* are all in the months April-July, and the four for *Stelgidopteryx ruficollis* all in April and May (Text-fig.5i). Doubtless the latter do not show the full extent of the breeding season. The only two *Stelgidopteryx* in moult, out of 14 trapped in four different months, were both trapped in August. From these records it is reasonable to conclude that the breeding season of *Progne*, and probably also *Stelgidopteryx*, is similar to, but perhaps a little earlier than, that of the swifts, and the same adaptive relationship with the food supply may be suggested.

Wrens (Troglodytidae)

Both species have long breeding seasons. *Troglodytes musculus*, a species associated with man and frequently nesting in houses, was found breeding in all months, with peaks in May and October (Text-fig. 5j), and *Thryothorus rutilus*, a forest species, from January to July, with a gap in May which is probably not significant.

A complete series of records kept for two or three pairs of *Troglodytes*, nesting in two houses in the Arima Valley over a period of 4½ years, showed less continuous breeding activity than is indicated by the combined records from all localities (Table IX). There was a marked peak in April-June, a complete gap in July-August, and a second more extended period of laying

Table VII. Breeding Seasons of Flycatchers

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
5 forest species	—	—	2	2	3	4	3	1	1	—	—	—
7 open woodland species	—	2	3	3	3	3	4	2	1	1	2	—
4 savanna species	2	4	4	4	4	4	3	2	—	1	2	2
2 swamp species	—	—	1	—	—	1	2	2	2	2	—	—

Note: Forest species: *Empidonax euleri, Platyrinchus mystaceus, Tolmomyias sulphurescens, Leptopogon superciliaris, Pipromorpha oleaginea.* Open woodland species: *Myiodynastes maculatus, Megarynchus pitangua, Contopus cinereus, Myiophobus fasciatus, Tolmomyias flaviventris, Myiopagis gaimardii, Camptostoma obsoletum.* Savanna species: *Tyrannus melancholicus, Legatus leucophaius, Pitangus sulphuratus, Elaenia flavogaster.* Swamp species: *Fluvicola pica, Arundinicola leucocephala.*

TABLE VIII. BREEDING AND MOULTING OF *Pipromorpha oleaginea*

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Breeding season	—	—	2	7	18	22	9	2	1	—	—	—
Number in moult, as a fraction of the total trapped	$\frac{0}{26}$	$\frac{0}{22}$	$\frac{0}{16}$	$\frac{0}{44}$	$\frac{1}{13}$	$\frac{1}{33}$	$\frac{2}{13}$	$\frac{6}{14}$	$\frac{5}{19}$	$\frac{5}{18}$	$\frac{0}{21}$	$\frac{0}{31}$

from September to January, followed by a nearly complete gap in February and March, the height of the dry season. The remainder of the records were nearly all collected by Trinidad Regional Virus Laboratory field assistants in the wetter Sangre Grande area, some 15 miles to the east. They are the only records that there was reason to suppose were biassed, since at times of the year when few other nests could be found (mainly in the months August-December), they concentrated on *Troglodytes*. But the records are interesting in showing that breeding is continuous, even though in this area too an unbiased sample might show peaks at the same time as in the Arima Valley.

Out of 24 *Troglodytes,* trapped in ten different months, and 25 *Thryothorus,* trapped in nine different months, only three birds were in moult, one *Troglodytes* in August and two *Thryothorus* in March and August. Both species sing throughout the year, but a marked resurgence of song was noted in *Troglodytes* in September. This is in accord with the July-August gap in breeding and the single moult record, and suggests that July and August are the main months of moult.

Mockingbird (Mimidae)

A fairly complete series of 41 records was obtained for three or four pairs of *Mimus gilvus,* the only representative of the family, nesting in gardens and orchards near the town of Arima over three years, and smaller numbers from other scattered localities (the species does not breed up the Arima Valley). The Arima records show a main period of breeding from January to July, with 88% of the nests in these months, and a minor period from September to November. The records from other localities also fall into these two periods in similar proportions (Text-fig. 5k). Though mockingbirds were not trapped, a break in July and August in the otherwise more or less continuous song-period, followed by a sudden revival of song in September, shows that the months July and August are almost certainly the height of the moult season, as in many other species. The double breeding season, with a minor peak following the moult and a major peak several months later, is essentially similar to the situation in *Troglodytes* in the Arima Valley, *Turdus albicollis* and *T. fumigatus* (below), *Thraupis palmarum* (p. 15), and perhaps other species, and is discussed in a later section (p. 23).

Thrushes (Turdidae)

The breeding seasons of the three *Turdus* species have been dealt with in detail in a previous paper (Snow & Snow, 1963). *T. fumigatus* and *T. albicollis* have very long breeding seasons, from later October or November to July or August, both with a minor peak in November and a major peak in June (Table X, Text-fig. 5l). The breeding season of *T. nudigenis* is much shorter, lasting from late April to August. Extensive trapping data for *T. fumigatus* and *T. nudigenis* showed that both moult at about the same time, the former in July-October and the latter in August-November. The song periods of all three species are co-extensive with their breeding seasons. The only plausible reason that suggests itself for the great difference between the breeding season of *T. nudigenis* and the other two species lies in their different habitat-preferences. *T. nudigenis* is a bird of semi-open country, where the effect of the dry season is more severely felt than in the forest, and the food supply for the young may be abundant enough only in the early part of the wet season. The other two are forest species, and so probably have a food supply that is less affected by the changing seasons. It may be noted that the two peaks in their long breeding season resemble those of *Mimus gilvus,* discussed above, and other species with very long breeding seasons.

Gnatcatchers (Polioptilidae)

Breeding records for the single species, *Ram-*

TABLE IX. BREEDING SEASON OF *Troglodytes musculus*

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Lower Arima Valley	4	1	—	3	6	4	—	—	2	5	4	2
All localities	5	5	2	8	11	10	5	4	7	13	7	8

TABLE X. ANNUAL CYCLES OF *Turdus* SPECIES

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Turdus albicollis												
Breeding season	1	6	10	9	11	20	3	3	—	—	7	4
Turdus fumigatus												
Breeding season	8	19	18	23	26	36	19	1	—	2	18	11
Number moulting, as a fraction of the total	$\frac{1}{15}$	$\frac{0}{8}$	$\frac{0}{5}$	$\frac{0}{4}$	$\frac{0}{11}$	$\frac{0}{16}$	$\frac{2}{8}$	$\frac{4}{7}$	$\frac{2}{5}$	$\frac{3}{8}$	$\frac{0}{8}$	$\frac{0}{11}$
Turdus nudigenis												
Breeding season	—	—	—	2	52	53	33	11	—	—	—	—
Number moulting, as a fraction of the total	$\frac{0}{6}$	$\frac{0}{3}$	$\frac{0}{7}$	$\frac{0}{9}$	$\frac{0}{7}$	$\frac{0}{10}$	$\frac{0}{13}$	$\frac{2}{13}$	$\frac{3}{3}$	$\frac{1}{2}$	$\frac{3}{8}$	$\frac{0}{21}$

phocaenus melanurus, of doubtful affinities but provisionally included in this family, are from February to August, and there is a single moult record in December. The information is too slender for generalizations.

Vireos (Vireonidae)

Records are few, especially for *Hylophilus.* The five records for *Vireo olivaceus* are restricted to April, May and June, but agree well with the restricted period of calling (usually early March to July), so are probably a satisfactory indication of the breeding season. There is a single moult record for early August. (The species appears to be a migrant to Trinidad, since we had no records of its occurrence from October to mid-February). *Cyclarhis gujanensis* apparently breeds rather late, as all our five records were from June to November, but our moult records were in the same period and more are needed to clarify its annual cycle.

Honeycreepers (Coerebidae)

Coereba flaveola, the bananaquit, is included in this section for convenience, although it is probably closer to the New World warblers (Parulidae) than to the rest of the honeycreepers. It is a much more specialized nectar feeder than the others. The large numbers of breeding and moult records for *Coereba* show that the total extent of its breeding season is long, from November to June (with a single August record), but that most breeding takes place from January to May (Table XI, Text-fig. 5m). This is a season very similar to that of the hummingbirds, the only other primarily nectarivorous birds, and the same adaptive relationship may be suggested. Also as in the hummingbirds, the moult takes place from May to October.

Rather few records were obtained for the four typical honeycreepers *(Chlorophanes, Dacnis* and *Cyanerpes* spp.), but they agree in showing a later breeding season, from March to July, and a mainly later season of moult, August-November. These species depend far less on nectar than does *Coereba;* correspondingly, their breeding seasons show a peak which coincides with that of the bulk of other land birds. Only two records were obtained for the rather distinct *Conirostrum bicolor,* a mangrove bird, and no generalizations can be based on them.

Warblers (Parulidae)

Again, records are few. *Basileuterus* certainly breeds mainly in the first half of the year, as our five records are from March to June and the main period of song is from late December to June. For *Geothlypis,* a swamp bird, information is scanty, but since three of the four very scattered records are from May to October and it sings more in the wet season than in the dry, it may have a mainly late breeding season like the other swamp passerines. We have no records for *Parula pitiayumi,* which sings throughout the year, but Belcher & Smooker give two, in June and July. We have no moult records for these three species.

TABLE XI. BREEDING AND MOULTING OF *Coereba flaveola*

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Breeding season	23	7	15	9	6	2	—	1	—	—	1	2
Number in moult, as a fraction of the total trapped	$\frac{0}{28}$	$\frac{0}{16}$	$\frac{0}{10}$	$\frac{0}{43}$	$\frac{1}{14}$	$\frac{2}{21}$	$\frac{5}{10}$	$\frac{7}{40}$	$\frac{2}{63}$	$\frac{1}{38}$	$\frac{0}{33}$	$\frac{0}{41}$

Orioles and Allies (Icteridae)

The high, hanging nests of the large oropendola, *Psarocolius decumanus*, were never reached, but from our own observations and those of others (Tashian, 1957; Drury, 1962) January-May can safely be taken to be the breeding season. The smaller cacique, *Cacicus cela*, breeds at the same period, while *Icterus nigrogularis* has a longer and slightly later season. All these species build elaborate hanging nests in exposed positions. Nest-building is probably hindered by rain and the safety of the nests, once built, is probably endangered by very wet weather, so that the rather exact synchronization of breeding with the dry season seems to have a clear adaptive basis.

The breeding season of *Quiscalus lugubris* is divided into two parts, with a major period in May-August and a minor period in November-February. A fairly complete series of 19 records from a limited area at Arima, collected over a period of three years, and 12 scattered records from other localities, both fall into these two divisions. *Agelaius icterocephalus*, a swamp species, has a late breeding season, from June to November, similar to that of the other swamp birds already mentioned.

Of the two parasitic species, one, *Psomocolax*, breeds at the same time as its hosts, *Psarocolius* and *Cacicus;* the other, *Molothrus*, which parasitizes a large number of diverse species, breeds from April to October.

We have few records of moult, except for *Agelaius*. Out of 43 *Agelaius* trapped in mid and late January, 23 were moulting, nearly all of them being in a very late stage of moult, and the rest were in fresh plumage. Only one out of 16 trapped in June and July was moulting, and this appeared to be a young bird undergoing a moult involving some inner secondaries only. Thus it appears that its moult season, like its breeding season, is late.

Tanagers (Thraupidae)

Large numbers of records were obtained for *Ramphocelus carbo*, *Thraupis palmarum* and *T. virens*, and adequate numbers for *Tachyphonus rufus*, *Habia rubica* and *Tanagra violacea*. Moult data were also obtained for all these species; so that for these six we can be sure that the picture of the breeding season and annual cycle presented here is substantially correct (Text-fig. 5n). For the remaining species the information is rather scanty, except that the trapping records of *Tangara gyrola* and *T. mexicana* were numerous enough to show their season of moult. The two breeding records for *Piranga flava* are chiefly of interest as representing an addition to the known breeding birds of Trinidad.

Ramphocelus carbo has a long breeding season with two well-marked peaks, in January and June. In each year, in the Sangre Grande area where most of the records were obtained, a burst of nesting began in early January, with sometimes one or two early nests at the end of December. Breeding then declined in February and March, then rose to its second peak. Late nests occurred until August, with one in September. Birds were trapped in moult over a long period (May-December), but 80% of them in the months July-October (Table XII).

The two *Thraupis* species also have long breeding seasons. Nests of *T. palmarum* were recorded in every month and nests of *T. virens* in eleven months of the year. But both show a well-marked and rather similar seasonal pattern, with a major peak (apparently double in *T. palmarum*) from March to July or August, and a small peak in October in the middle of a five-month period (August-December) when relatively few nests are started.

For 4½ years, a complete record was kept of the nesting of *T. palmarum* in the crowns of three ornamental palms at Simla, in the Arima Valley. The 35 nests thus recorded show almost exactly the same seasonal distribution as the combined records from all localities; 29 of them (83%) were in the months March-July. They are further discussed on p. 23.

Moulting individuals of *T. virens* were trapped in the three months June-August, and the moulting *T. palmarum* over a longer period, from May to December (Table XII). The difference would probably be reduced by further records, but is probably genuine and related to the difference in the extent of their breeding seasons,

The three species for which less abundant data were obtained, *Tachyphonus rufus*, *Habia rubica* and *Tanagra violacea*, all have broadly similar breeding seasons, with a peak in May-June (sharper in some than others), little breeding in the four preceding months, and little or none from September to the end of the year. In all three, moulting birds were caught only in the months June-October.

The annual cycles of the *Tangara* species do not conform to the pattern which, with wide variations, is nevertheless general to the preceding six species. The few breeding records would not of themselves suffice to indicate anything unusual—nests in all months except February, September, November and December (months in which breeding activity is reduced in the other species); but the moulting regime is anomalous. In the first place, the season of moult is very early, beginning in April and being at its height

TABLE XII. ANNUAL CYCLES OF TANAGERS

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Thraupis palmarum												
Breeding season	3	4	21	20	15	25	16	12	1	3	1	1
Number in moult, as a fraction of the total trapped	0/3	0/2	0/3	0/1	1/7	1/5	0/8	3/4	0/0	2/2	1/8	1/6
Thraupis virens												
Breeding season	8	4	10	31	21	20	11	1	—	3	1	1
Number in moult, as a fraction of the total trapped	0/2	0/0	0/6	0/4	0/2	2/6	3/5	7/9	0/3	0/0	0/5	0/3
Ramphocelus carbo												
Breeding season	33	14	14	19	20	35	14	9	1	—	—	3
Number in moult, as a fraction of the total trapped	0/25	0/8	0/12	0/17	2/28	5/24	6/32	23/35	16/26	4/15	4/12	1/36
Tachyphonus rufus												
Breeding season	—	1	1	2	6	7	2	1	—	—	1	—
Number in moult, as a fraction of the total trapped	0/9	0/2	0/2	0/10	0/10	0/8	0/3	3/6	4/4	1/8	0/4	0/8
Number of tanager species breeding in each month	6	5	7	8	8	8	9	8	2	5	3	3
Number of species in moult in each month	—	—	—	2	3	6	4	9	6	4	3	2

from May to July, at the same time as the breeding season appears to be at its height (Text-fig. 5n). Furthermore, the individual moult seemed to be a much slower process in *Tangara* than in the other tanagers studied, and in *T. gyrola* there was evidence of "arrested" moult, as was also found in *Columbigallina talpacoti*. These points are discussed in more detail in a later section (p. 22).

Swallow-tanager (Tersinidae)

Belcher & Smooker give a June record for the single species, *Tersina viridis*. We have seen two pairs entering and leaving nest-holes in May, as though nesting or preparing to nest. The species appears to be migratory, as it is in northern Venezuela (Schaefer, 1953), since all except one of the records of its occurrence known to us are in the months March-June. We have no moult records.

Finches and Seed-eaters
(Fringillidae and Emberizidae)

As a group, the small finches and seed-eaters breed mainly later than most other land-birds with the exception of the swamp species. Of the 80 nests recorded, 64 were in the months May-September, and the remaining 16 all in the following four months. (Table XIII, Text-fig. 5o). There seems little doubt that their season is related to the seeding of the grasses, which is at its height some time after the rains start, in May.

In *Volatinia*, for which there are most records, moulting birds were trapped in every month from August to February (with the exception of September, when only one bird was trapped); in the months March-July, none of the 32 birds examined was in wing moult. The fewer records that were obtained for *Sporophila* species showed moult in most months from June to February, and none in the months March-May.

Saltator coerulescens, a much larger and ecologically distinct species, probably feeding much on insects and to some extent on soft fruit, has a breeding season similar to that of the tanagers, at its height from April to July, and moults from August to January.

LOCAL DIFFERENCES WITHIN TRINIDAD

The considerable differences in climate within Trinidad would be expected to be associated with corresponding local differences in breeding season. In particular, breeding seasons would be expected to be shorter and more confined to the wet season in the northwest part of the island, where the rainfall is low and the dry season correspondingly severe, than elsewhere. As already mentioned, the bulk of the data on which this paper is based were collected in the Arima Valley in the central part of the Northern Range, near Arima itself, and in the Sangre Grande area some 15 miles to the east. Consequently we have not enough records from different areas for a proper examination of local differences, but such

TABLE XIII. BREEDING AND MOULTING OF SEEDEATERS

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Number of species breeding	2	—	—	—	3	5	5	4	4	1	3	3
Number of species moulting	2	2	—	—	—	3	—	2	1	1	2	3

as they are, they provide some evidence for the differences that would be expected.

As mentioned earlier, *Troglodytes* was found nesting in all months in the Sangre Grande area, but not in the Arima Valley, where its season was divided into two main periods, with a period of inactivity in the middle of the dry season and another in July-August. Likewise, *Columbigallina talpacoti* was found breeding almost continuously in the Sangre Grande area, but at Arima it ceased for the last few weeks of the dry season.

In each of the three years when nests were searched for, *Manacus* was found to start breeding considerably earlier in a valley about ten miles to the east than in the Arima Valley, in one year about six weeks earlier (Snow, 1962a). Wet weather is one of the external factors stimulating breeding in *Manacus,* as in other species (p. 25), while drought inhibits breeding, so that it is likely that towards the dry western end of the Northern Range there is an increasing tendency for birds to delay breeding until the onset of the wet season.

Breeding probably begins earlier at high altitudes in the Northern Range than in low-lying areas near by. Belcher & Smooker make the same generalization, but without supporting evidence; we had evidence only for *Elaenia flavogaster.* Of ten nest records from 1,200-1,800 ft. in the Northern Range, one each was in November, December and February, and the rest in April-June. None of the other 29 records from low altitudes was earlier than March. The reason for this difference remains obscure.

We had no breeding records from the very dry islands off the northwest corner of Trinidad, but visits at several times of year gave evidence of almost complete quiescence at the height of the dry season, followed by an outburst of song in April, just before the onset of wet weather. The state of the vegetation also suggested that, except perhaps for hummingbirds and *Coereba,* breeding in these islands must be mainly confined to the wet season. Since a day of intensive trapping in mid-October on Chacachacare, the driest and westernmost island, showed that many birds were in moult, as elsewhere at that season, we conclude that for most birds the breeding season there is short, lasting from May to September.

DIFFERENCES ACCORDING TO HABITAT

We have no data enabling us to test the effect of habitat on the breeding season of any individual species, except for the hummingbirds *Glaucis hirsuta* and *Amazilia tobaci.* As already mentioned, we found that both these species began breeding earlier in open places than in the forest. At least in *Glaucis,* the difference appeared to be related to the later appearance in the forest of the flowers on which it fed.

But interspecific comparisons afford some, though rather weak, grounds for supposing that breeding seasons are longer in open and semi-open country than in the forest, as has been found elsewhere in the tropics (Tanganyika, Moreau (1950); British Guiana, Davis (1953)). In the first place, all the species found to have continuous breeding seasons are characteristic of open habitats (*Columbigallina talpacoti, Crotophaga ani, Troglodytes musculus, Thraupis palmarum*). Differences in breeding seasons within two of the largest families, the flycatchers and tanagers, also provide some supporting evidence. As already mentioned (p. 12), the flycatchers of open country tend to have longer breeding seasons than the forest species. The tanagers most characteristic of open country, *Thraupis palmarum, T. virens* and *Ramphocelus carbo,* have longer breeding seasons than *Habia rubica* and *Tanagra violacea,* primarily forest species. However, inadequate knowledge of the breeding seasons of the species most strictly confined to forest (*Tangara gyrola, T. chrysophrys, Thraupis cyanocephala* and *Piranga flava*) makes this conclusion doubtful. The breeding seasons of the *Turdus* species run contrary to the generalization, *T. nudigenis,* the savanna species, having a much shorter season than *T. fumigatus* and *T. albicollis,* the two forest species. As pointed out earlier, there is an obvious reason why ground-feeding birds like thrushes should be able to breed for a greater part of the year in the forest than in open country. Finally, almost complete ignorance of the trogons and most of the antbirds, preeminently forest species, further weakens any generalization, especially as the little evidence we have suggests that they have very long breeding seasons.

If all species are taken together, the difference between the breeding seasons in forest and the various types of open and semi-open country

Table XIV. Breeding Seasons of *Pipromorpha oleaginea* in Five Years
(Number of nests started in half-monthly periods)

	1957	1958	1959	1960	1961
February 1				(B)	
February 2				(2B)	
March 1					
March 2		1			
April 1				(B)	
April 2		3	2		
May 1	1	1	4		
May 2	2		5	3	
June 1	4	1	4	2	3
June 2	2		4		2
July 1			1	2	2
July 2	1				2
August 1	1				1
August 2					
September 1	1				

Notes: Nests from the Arima Valley only are included. B: nest being built, but abandoned before completion.

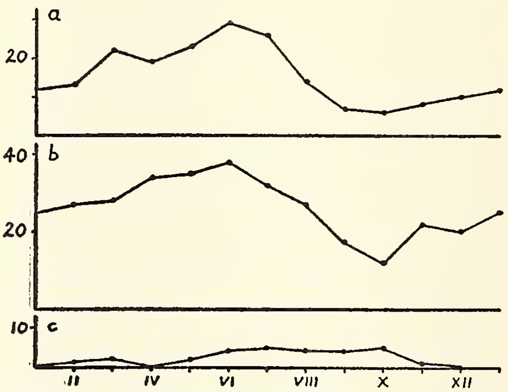
(except swamps) is not striking (Text-fig. 6). The only difference in breeding season according to habitat which is outstanding has already been mentioned and discussed in earlier sections; as Text-fig. 6 shows, the peak of breeding of the swamp species is from June to October, about three months later than the peak in other habitats.

Annual Differences in Breeding Season

Most species for which adequate numbers of nests were found in two or more years showed rather little annual variation in their breeding season. Thus no significant variation was found in the onset or duration of the breeding season in the swifts *Chaetura brachyura* and *Cypseloides rutilus* in five years, the thrushes *Turdus fumigatus* and *T. albicollis* in five years, or in *Coereba flaveola, Turdus nudigenis* and *Ramphocelus carbo* in three years, though larger numbers of records would doubtless have revealed small differences. In all of these, the breeding season in each year was much the same as for all years combined. The same was probably true of other species of which few nests were found in any one year, but whose song periods were rather constant from year to year *(e.g., Xiphorhynchus guttatus).* In the hummingbird *Glaucis hirsuta,* the time of onset of breeding varied by about a month in different years, and the beginning of the song periods of *Phaethornis longuemareus, Amazilia chionopectus* and *Colibri delphinae* showed a similar amount of variation. (For *Glaucis,* the comparison here is between birds nesting in the same habitats; it has already been mentioned that nesting begins later in forest than in open habitats).

The greatest annual variation in breeding season was found in *Manacus.* In the Arima Valley, the date of onset of breeding varied by five months, from early January to early June, in the five years of study (Snow, 1962a). The proximate factors involved were not clear. The beginning of breeding bore no fixed relation to changes in weather, though severe drought was found to inhibit breeding. It seemed that varying feeding conditions also exerted an effect. *Manacus* normally begins to breed during a period of increasing dryness and gradually increasing fruit supply, but conditions vary from year to year. It seems that in the Arima Valley the inhibiting effect of drought and the stimulating effects of increasing food supply are nicely balanced, so that slight changes in either can lead to large differences in the onset of breeding.

Although records were rather few in each year, *Pipromorpha oleaginea* also showed rather large variations in the beginning of breeding in the five years of study (Table XIV); in each year, the period of calling of the males agreed so closely with the periods when nests were found that there is little doubt that the observed differences are reliable. The variations agreed to a limited extent with those in *Manacus* in the same years. Thus in 1960, which was a very early season for *Manacus, Pipromorpha* started to call and build nests much earlier than in any other year, but none of the nests that were found were completed and there was an inactive period of about a month before nesting began again. *Pipromorpha* and *Manacus* would be expected to show some agreement in their response to proximate factors affecting breeding, as they occur in the same habitats and both nest mainly along streams, and both feed on fruit and insects,



Text-fig. 6. The breeding season in (a) forest, (b) open and semi-open country, and (c) swamps; as shown by the numbers of species found breeding in each month.

though *Manacus* is much the more specialized fruit-eater.

In view of the variability in *Pipromorpha's* breeding season, it is of especial interest that individuals were found to show very little variation in the dates on which they began to moult in different years (p. 23). In this they differed from *Manacus*, which showed much greater annual differences in the moult dates of individuals, though not nearly such great differences as in the time of onset of breeding.

In *Steatornis*, the only other species for which we have data, the date of onset of breeding was found to vary by about three months in different years, from December to late March (Snow, 1962 (1963)d). No satisfactory explanation could be suggested for the particular differences which were found, but two considerations are relevant. The nesting cycle of this species is very long, so that it may be impossible for a pair, if it has had two broods in close succession, to nest again at the same time in the following year; and since the species is highly social, the start of breeding tends to be synchronized in a colony.

Number of Broods per Year and Intervals Between Broods

In nearly all the species for which we have information, a complete nesting cycle, from nest-building to the fledging of the young, is far shorter than the extent of the annual breeding season. In most, several complete nesting cycles could be fitted into the season, and it is of interest to know how many nesting attempts are in fact made. Without such information we cannot fully understand the tropical breeding season, nor compare the reproductive rate of tropical species with their counterparts in temperate regions. We were able to collect data on this head for several species, especially those that habitually re-use the same nest or build another close to the previous one.

Turdus species

In the thrush *Turdus fumigatus*, on the basis of re-use of nests and also from observations on color-ringed birds, it was found that three or four broods may be reared in a year (Snow & Snow, 1963). In the months April-July, relaying followed rather soon after the end of the previous nesting attempt, or else there was a very long gap, doubtless because the moult intervened. In the months December-February, the intervals were mostly rather long, but variable, corresponding to the erratic and low incidence of breeding during the dry season (Table XV).

In *T. nudigenis*, whose breeding season is only three months long, there were two records of the re-use of the same nest, the intervals being 10 and 15 days. The breeding season is hardly long enough for more than two broods to be reared. Haverschmidt (1959) gives records of four broods reared in the same nest of *T. leucomelas* in Surinam, where the species has a breeding season of about eight months, the intervals being 18, 8 and 12 days.

The shortest intervals between broods, of 15 days or less, are of about the same length as the usual intervals in the European Blackbird *(T. merula)*, which usually makes 2-4 nesting attempts within a breeding season of about four months (Snow, 1958). *T. nudigenis*, with a breeding season of about three months, almost certainly makes on average fewer nesting attempts than *T. merula* in England, while *T. fumigatus*, because of the very long intervals between broods in the dry season, makes about as many attempts in spite of having an almost continuous breeding season.

Manacus manacus

In *Manacus*, the breeding season varied in length from four to eight months in the five years under study. Each female made as a rule two or three nesting attempts in the shorter seasons, and more often three or four in the longer seasons. Intervals between broods were variable, (Table XV), at least partly in relation to favorable or unfavorable environmental conditions (Snow, 1962a).

Thraupis palmarum

For this species, the intervals between successive nesting attempts could be determined for the birds, already mentioned, that nested (one pair per tree) in three ornamental palm trees in the Arima Valley. Of the 20 intervals, 12 were between 11 and 25 days, and the other eight all much longer (Table XV). Nesting was especially successful in one of the trees, which had 19 of the 35 nests. From Text-fig. 7, which shows the succession of nests in this tree graphically, it will be seen that there was generally a rather quick succession of nesting attempts from March to August, then a gap during which the birds presumably moulted (this being the moult season for the species). In three of the four years there was then a single nesting attempt in October or November, followed by a rather long gap until the next attempt in February or later. The seasonal distribution of nests in this one site matches that of the combined records quite closely, so that the intervals too are probably typical of the species as a whole.

Troglodytes musculus

For *Troglodytes*, six intervals were deter-

TABLE XV. INTERVALS BETWEEN SUCCESSIVE BROODS IN TWELVE SPECIES

	Number of days between ending of one nesting attempt and laying of first egg of next clutch									
	0	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	41+
Columbigallina talpacoti	—	1	1	1	—	—	1	1	—	—
Steatornis caripensis	2	—	—	—	3	6	—	1	—	35
Chaetura brachyura	—	1	3	3	5	3	3	5	—	4
Cypseloides rutilus	1	—	4	2	—	1	—	—	—	—
Glaucis hirsuta	—	—	—	1	1	6	1	3	1	1
Anthracothorax nigricollis	—	—	1	—	1	1	1	—	1	1
Amazilia tobaci	—	—	—	1	1	—	1	—	—	—
Manacus manacus	—	1	1	7	3	6	2	6	4	9
Troglodytes musculus	—	—	—	—	—	—	—	—	—	6
Turdus fumigatus	—	—	3	2	4	—	1	2	1	8
Turdus nudigenis	—	—	1	1	—	—	—	—	—	—
Thraupis palmarum	—	—	—	5	3	4	—	—	—	8

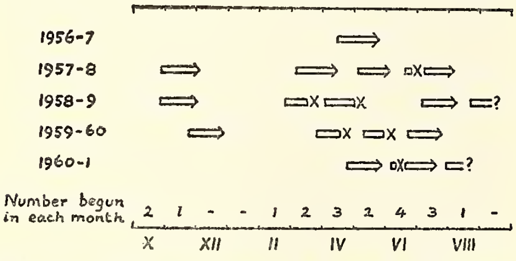
mined for a pair which nested behind the ventilator above a window for over four years, from March 1957, to September, 1961. It was not certain that the same female survived for the whole of this period; in fact there was probably a change of female in early 1959; but it is safe to assume that most of the intervals are between successive nestings of the same female, and certainly that there was continuity of one member of the pair between every interval. The sequence of nests was rather similar to that of *Thraupis palmarum* in that in each year the birds started to nest between late October and early December, presumably soon after they had finished moulting. But thereafter the intervals between nests were much longer, especially in the middle of the dry season; there was none of less than 40 days, and only two or three nesting attempts were made in the year (3, 2, 2 and 3 in the four years). The sequence is shown graphically in Text-fig. 8.

Hummingbirds

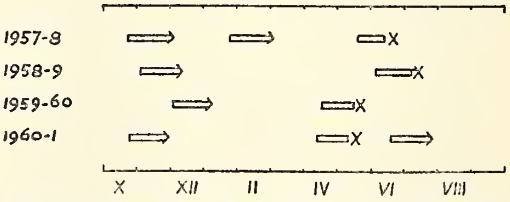
Information is available for three species of hummingbirds. *Anthracothorax nigricollis* was found to nest two or three times in the season (five records; 2, 2, 3, 3, 3), and for *Amazilia tobaci* there was one record of three successive broods and several of two broods in the season. For both species, the intervals between broods were variable, but few of them were very short (Table XV). For *Glaucis hirsuta,* 14 intervals were recorded between broods in the same nest, and these also were variable and mostly rather long. Because of the abundance of this species along the streams where it nests, the building of a new nest very near to an old one that had been used shortly before could not safely be ascribed to the same bird, so that the number of nesting attempts per year could not be ascertained, but 2, 3 or occasionally 4 seemed to be the rule.

Swifts

Chaetura brachyura, the species for which most information was collected, was found to make from one to three, occasionally four, nesting attempts within the breeding season, which lasted about four months (Snow, 1962b.) Nest losses were high in the colony under study and the number of nesting attempts was almost certainly higher than it would be in a more successful breeding population. None of the pairs which were successful with their first brood



TEXT-FIG. 7. The succession of nests of *Thraupis palmarum* in a single palm tree, 1957-1961. Each nesting attempt is represented by an arrow, if successful, or by a rectangle followed by an X, if unsuccessful.



TEXT-FIG. 8. The succession of nests of *Troglodytes musculus* in a single nest-site, 1957-1961. Conventions as in Text-fig. 7.

made more than one further attempt in the same season. The situation was similar in *Cypseloides rutilus*, but breeding success was higher and the average number of nesting attempts consequently lower, although intervals between successive nesting attempts were on average shorter (Table XV).

Steatornis caripensis

The nesting regime of *Steatornis* is peculiar (Snow, 1962 (1963)d). Each nesting cycle is very long, lasting nearly six months. Two may just be completed within one year, and there were a few records of this and even of three being completed in 18 months. These quick successions were then followed by long intervals of many months.

During the months when most clutches were laid (December-May), it was usual for laying to follow rather quickly after the ending of a previous nesting attempt, giving the short intervals shown in Table XV, but nesting attempts ending in the other months of the year were usually followed by intervals of four months or more.

Columbigallina talpacoti

For this dove there was a record, unfortunately unsupported by precise dates, of a succession of five broods in the same nest, and three records of two broods being reared in the same nest. In addition, Junge & Mees (1958) record a succession of three broods in one nest. Three of the five known intervals between successive attempts were short (2, 10, 14-15, 28 and 33 days). For the same species in Surinam, Haverschmidt (1953) records intervals of 13 and 34 days. These intervals suggest that within its almost continuous breeding season there tend to be quick successions of broods during favorable periods, followed by long intervals when conditions become less favorable. The opportunist nature of this dove's breeding is discussed elsewhere in relation to its breeding season and moult (p. 5).

To summarize, all the species discussed above regularly make more than one nesting attempt in the course of the breeding season. The number of attempts depends partly on the length of the breeding season, but is by no means proportional to it, since in those species with very long breeding seasons there tend to be very long intervals between broods at certain times of the year. At least in some species (*T. fumigatus, Manacus, Troglodytes*), these long intervals occur at times of year which there is reason to suppose are relatively unfavorable for breeding; their effect is to produce a "trough" in the pattern of the breeding season, while the short intervals are associated with the "peaks."

Duration of the Moult in the Individual

There is little information available as to how long it takes for an individual bird of any species to complete its moult under natural conditions. For tropical land-birds, there seem to be only Miller's records for the Andean Sparrow *(Zonotrichia capensis)*, which completes its moult in about two months with rather little variation either way (Miller, 1961); while Baldwin (1953), from a study of skins, suggested five months as the likely duration of moult in Hawaiian honeycreepers (Drepanididae). In the Oilbird, the moult almost certainly lasts at least six months (Snow, 1962 (1963)d), a period comparable to that taken by sea-birds (*e.g.*, 7 months for *Anous stolidus*—Ashmole, 1962). With such wide potential variation, it is important, for an understanding of the annual cycle, to have some idea of the usual duration of the moult in the species with which we are concerned here, especially those with very long or continuous breeding seasons.

Repeated trapping of the same individuals gave us some information on this head for eleven species. The fullest data are on *Manacus*, of which seven individuals were trapped more than once in the course of a single moult. From the progress made between successive trappings, it was found that, except for the innermost and outermost feathers, two or three of which drop more or less together, the primaries drop at intervals of 8-10 days and the complete replacement of the primaries must take about 80 days (Snow, 1962a). This agreed exactly with the length of absence of individual adult males, while moulting, from their courts at the display ground, which was known accurately in nine cases to be between 76 and 85 days.

Less complete information was obtained for several other species, and is given, with that for *Manacus*, in Table XVI. Except for *Steatornis*, which is exceptional in the slowness of its moult as of its breeding, the figures show good general agreement. In the two piciform species, *Celeus elegans* and *Momotus momota*, the progress of primary moult was at the rate of one feather per 16 days, while in the passerines the rates were mainly around one feather per 8-10 days, giving estimated total durations of around 90 days. This agrees well with our only two records for complete moult periods. An individual of *Turdus fumigatus*, trapped just as it was beginning its moult, had very nearly finished it 91 days later, and an individual of *Pipra erythrocephala*, trapped when it was just beginning to moult, had completed its moult 96 days later.

Table XVI. Rate of Progress of Wing Moult in Ten Species

	Number of primaries replaced between captures	Primaries replaced (numbered from inside)	Interval in days	Rate of moult: number of days per primary
Momotus momota	6	2-7	96	16
Celeus elegans	4	3-6	65	16
Xiphorhynchus guttatus	2	3-4	14	7
Dendrocincla fuliginosa	3	1-3	45	15
Manacus manacus	9	1-9	79	9
Manacus manacus	7	3-9	59	8
Manacus manacus	3½	1-4	27	8
Manacus manacus	3	3-5	25	8
Manacus manacus	3	4-6	25	8
Manacus manacus	1¾	3-4	21	12
Manacus manacus	1½	1-2	12	8
Pipra erythrocephala	3	3-5	20	7
Pipra erythrocephala	1¾	2-3	13	7
Pipromorpha oleaginea	2	6-7	19	10
Turdus fumigatus	3½	6-9	50	14
Tachyphonus rufus	3	3-5	22	7
Ramphocelus carbo	5	2-6	73	15
Ramphocelus carbo	3½	1-4	28	8
Ramphocelus carbo	2	2-3	28	14

Other things being equal, the greater the number of adjacent primary feathers being replaced at the same time, the more quickly will the wing-moult be completed. In fact, in many species considerable individual variation was found in the number of adjacent feathers growing at the same time, and for all individuals the number tends to be larger in the early and late than in the middle stages of the moult. In *Manacus* and *Pipra,* two or three were the usual numbers. In *Celeus,* whose moult probably takes longer, one or two adjacent feathers only were found growing at the same time. In *Thamnophilus* and *Columbigallina talpacoti* it was unusual for more than one primary in each wing to be growing at the same time. It will be recalled that in these two species both the breeding and moult seasons are more or less continuous, and there is doubtless a connection between the ill-defined annual cycle and the fact that their moult is a slower process than in species with better defined annual cycles.

Tanagers of the genus *Tangara* also seem to have a very slow moult, and in this respect, as in their season of moult (p. 15), to stand somewhat apart from their relatives. In them too it was found to be unusual for more than one primary to be growing at the same time in each wing (one primary in ten instances, two primaries in three instances); whereas in other tanagers, though there is considerable variability, it was more usual to find two adjacent feathers growing simultaneously than any other number, and three or four were not uncommon. In *T. gyrola,* the only individual that was trapped twice while moulting gave evidence of a very slow rate of moult. The two primary feathers that were growing at the time of the first capture (6th primary in each wing, ¾-grown and nearly full-grown respectively) were still not full-grown 15 days later and no other feathers had fallen. (This individual is omitted from Table XVI, as the rate of moult cannot be calculated in this case). In addition, two individuals of *T. gyrola* showed a condition of arrested moult similar to what was found in *Columbigallina talpacoti* (p. 5). However, in spite of this evidence for a slow rate of moult, the total duration of the moult season of *Tangara* species is not particularly long (Text-fig. 5n), which suggests that the moults of the individuals within the population may be better synchronized than in the other tanagers. Clearly the annual cycle in *Tangara* would repay more detailed study.

Individual and Annual Variation in the Season of Moult

If about three months must be allowed for the moult in typical small or medium-sized passerine birds, and longer periods for the larger birds, as the above figures suggest, a continuous or nearly continuous breeding season is possible only if the annual cycles of the different individuals in the population are not very exactly synchronized. An alternative possibility is that individuals may moult while breeding, but our evidence suggests that in at least most of the species with which we are concerned this is not so. We had enough re-traps of different individuals moulting in the same year, and of the same individuals during their moults in different years, to show that there is, as would be expected, enough individual and annual variation to account for the overlapping breeding and moulting seasons that were in fact found.

Nine individuals of *Manacus* were trapped while moulting in two or more different years. They showed variations of up to 78 days in the calculated dates of onset of their moults in different years, though in five of the eight comparisons that could be made between successive years the difference was 26 days or less. The dates on which color-ringed adult males disappeared from their courts at a display ground for their moult, and re-appeared after the moult, showed similar annual variations (Snow, 1962a). Records for four individuals of *Pipra erythrocephala* showed smaller annual variations—a maximum difference of 39 days between different years, and differences of 12, 21 and 26 days between successive years.

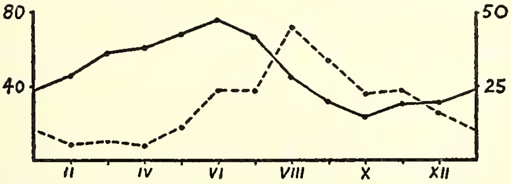
The records for four individuals of *Pipromorpha oleaginea* show surprising constancy from year to year, the calculated dates of the start of moult being as follows:

	1958	1960	1961
Bird no. 1	July 4	July 18	July 11
Bird no. 2	July 4	—	July 13
Bird no. 3	—	Aug. 7	Aug. 11
Bird no. 4	—	June 27	June 28

In addition to these three species, single individuals of five other species were trapped in moult in two successive years: *Xiphorhynchus guttatus, Myrmotherula axillaris, Leptopogon superciliaris, Dacnis cayana* and *Tachyphonus luctuosus.* Allowing for the probable rate of moult, all proved to have started their moults at about the same date in both years.

THE GENERAL PATTERN OF THE ANNUAL CYCLE

Text-fig. 9 shows the breeding and moulting seasons of all the species combined, by summation of the numbers of species found breeding and moulting in each month. Of course the picture produced by such treatment of the data is of limited significance, though for lack of any better means it has had to be used in many studies of tropical breeding seasons. In the first place, even if one's information were complete for every species, the pattern so obtained would not correspond to any one actual breeding or moulting season, being derived from numerous species with widely varying seasons. Secondly, the information is in fact far from complete, so that the shape of the pattern is much affected by chance variations in the contributions made by the different species, according to their abundance or rarity and the ease with which they can be trapped or their nests found. Thus if the information were much better for the hummingbirds than for the seed-eaters (as is the case), the breeding curve would be too high in the early months of the year.



TEXT-FIG. 9. The general breeding season and moulting season of Trinidad land-birds. Solid line: number of species found breeding in each month. Broken line: number of species found moulting in each month.

Accepting these limitations, we can nevertheless safely take the months April-June as the height of the breeding season in Trinidad. In these months nearly all the species of land-birds are breeding—the early ones have not quite finished, the late ones are just starting, and most of the others are in the middle of their breeding season. To this can be added the further generalization, that moulting is at its height in the months July-October.

Whether, within this broad pattern, any individual species or group is early or late, or has a long or a short breeding season, can in many cases be explained on the basis of its ecological requirements. The evidence on this head for the oilbird, hummingbirds, swifts and swallows, nightjars, manakins, bananaquit, icterids and seed-eaters has already been given in the preceding sections. The swamp-inhabiting species, as a group, are another good example; the reason for their late breeding season has also been discussed (p. 12).

There remain a number of species with continuous or nearly continuous breeding seasons. A few, such as *Columbigallina talpacoti, Crotophaga ani* and *Thamnophilus doliatus,* appear to be emancipated from a fixed annual cycle and to both breed and moult in any month. But most of them, as far as known, moult at the same time of year as the other species. This is certainly so for the tanagers *Thraupis palmarum* and *T. virens* and the thrush *Turdus fumigatus,* and probably so for *Pitangus sulphuratus, Turdus albicollis, Troglodytes musculus* and *Mimus gilvus.* It is difficult to believe that the single month or two in which these species do not breed is in some way unsuitable for breeding. The most reasonable explanation is that environmental conditions are suitable for breeding at all times of year, but that the birds have kept to an annual cycle which is adapted to conditions prevailing in neighboring areas with more seasonal climates.

The very long breeding seasons of the species mentioned above also have in common the fact that they are bimodal. Soon after the end of the moult, or the time when it may be presumed to have ended, there is a resurgence of breeding, reaching a peak usually in October or November. This is then followed by a decline, which in its turn is followed by a more gradual increase in activity leading to the second, main peak in May or June. The period of reduced activity, in all the species, coincides roughly with the first part of the dry season, but the decline itself cannot be a direct result of dry weather since it begins while the weather is still very wet, in November or December. Furthermore, the resurgence which leads to the May peak begins while the dry season is at its height. It seems likely that this double-peaked breeding season depends mainly on an internal rhythm, and is perhaps accentuated by the inhibiting effect of dry weather in January-March and low minimum temperatures in November-February (see also p. 26).

Reviewing the general pattern of the annual cycle of breeding and moult, one cannot fail to be struck by certain broad similarities between the annual cycles of Trinidad birds and those which prevail in the northern hemisphere generally. The peak of breeding is at approximately the same time, the moult is at much the same time, and there is an apparent analogy between the minor October-November peak of breeding in Trinidad and the autumn recrudescence of sexual behavior in northern birds. One is led to wonder whether these main events of the annual cycle in Trinidad are in fact "homologous" with the events in the north-temperate region in the sense that they pass gradually into one another in the intervening latitudes, and if so, to what extent they are controlled by the same proximate and ultimate external factors. These and related problems will be discussed in the following sections.

ULTIMATE FACTORS CONTROLLING BREEDING

In discussing the factors controlling breeding seasons it is helpful to distinguish between ultimate and proximate factors, as originally pointed out by Baker (1938). Ultimate factors are those which make breeding possible, or more likely to succeed, at one time of the year rather than another, and include especially, for birds, weather and the food supply for the young. These are the factors that ultimately determine at what time of year breeding takes place. Proximate factors are those factors in the changing environment to which the organism responds, and which act as the timers of the breeding season in the physiological sense. They need be themselves of no direct significance for breeding. The shorter the period between the beginning of breeding activities and the production of young, the more likely it is that ultimate and proximate factors will be closely related. Thus for a small bird the proximate factor may be rainfall, and the ultimate factor may be the increased food supply which immediately follows rainfall.

It has been shown for two specialized fruit-eaters whose diets show no overlap, *Steatornis* and *Manacus*, that most young are in the nest when the greatest variety of fruit is available, and in both species this period is in the months April-June. However, since their incubation and fledging periods are very different, their egg-laying seasons differ greatly. Two swifts, among the most specialized of the insectivores, start to breed at the end of April or early May, and there is good reason to believe that the early part of the wet season, from mid-May onwards, when most of their young are in the nest, is the period when insect life is at its most abundant. Thus both for specialized fruit-eaters and specialized insect-eaters the greatest abundance of food seems to be available in the same months of the year, and it is not surprising that this is the time when the breeding season of the land-birds taken as a whole reaches its main peak. Nor is it difficult to see the selective advantage gained by certain groups, of specialized habitat, diet or nesting habit, in breeding earlier or later than the others. These have been discussed in detail in earlier sections.

Hence it is a reasonable hypothesis that the breeding seasons are ultimately adapted to take advantage of the periods of greatest food supply and, in some species, to the period of greatest safety of the nest. That the seasons, thus evolved, reach their height in the main at the same time of year as the breeding season in the north-temperate region, can be regarded as a biological coincidence, resulting from the fact that the north-tropical wet season, determined by the northward movement of the equatorial low-pressure belt, and the northern spring, begin at about the same time. Both, of course, are determined by the northward movement of the sun's declination.[2]

The fact that the moult season in Trinidad shows a similar correspondence with the moult season in the north is more puzzling. Beginning as it does approximately in the middle of the wet season, it seems to restrict the breeding season unnecessarily. Those species which wait for the wet season before breeding would do better, one would suppose, to continue breeding longer and moult later. As it is, the length of the breeding season of each species seems to depend on how soon it can begin to breed before the wet season starts. Some, like *Turdus fumigatus,* begin as soon as the moult is over, in October or November of the previous year; others, like *T. nudigenis,* not until the following May.

No final explanation is possible at present, but it may be that in most habitats the food supply declines significantly, and perhaps sharply, in the course of the wet season, so that conditions for rearing young may deteriorate even though the general aspect of the environment is unchanged. That this is indeed so for the fruit-eating birds is suggested by the data for both *Manacus* and *Steatornis.* The variety of fruits eaten by *Manacus* was found to reach its lowest point in September, and for *Steatornis* the availability of fruits of Lauraceae, the main family on which the young are fed, declines sharply in July. For insectivores we have no equivalent data, but it is perhaps significant that in swamps, where the food supply might be expected to reach its peak later than in other habitats, the insectivorous land birds both breed and moult later than those in other habitats.

PROXIMATE FACTORS

Rainfall

For most species, we can say little about the proximate factors controlling their breeding. Indeed, we were impressed during our field work by the way in which species would start to breed and be well synchronized, unassociated with any clear environmental change. The few indications that we had were connected with the onset of the wet season. Discussion of other environmental factors is mostly speculative.

In 1959, very dry weather prevailed until April 9. Heavy rain fell on the 9th and 10th and immediately afterwards birds of several species were seen carrying nest-material. The first eggs of the season of *Manacus* were found ten days later, and four nests of *Turdus fumigatus* had eggs 9-13 days later, after a gap of nearly two months during which none had been found. In 1961 the wet season began late. There was some rain on May 22, but it then became very dry again for a week and wet weather did not properly start until May 28. As in 1959, it was followed by an outburst of breeding. The first eggs of *Turdus fumigatus,* which had stopped breeding and singing for several weeks, were recorded on June 6, and *Manacus,* which had practically stopped breeding since April, had a great resurgence of egg-laying in early June.

In other years, the first wet weather in April or May was not followed by any conspicuous outburst of laying. It can hardly be doubted that the more seasonal the climate and the more severe the dry season, the more important rainfall is likely to be as a proximate factor stimulating breeding. Many studies in arid regions have shown this, and in northern Venezuela, only a short distance to the west of Trinidad but with a considerably more seasonal climate, Gilliard (1959) describes how the onset of the rains has a spectacularly stimulating effect on breeding. It is reasonable to conclude that in the Arima Valley, and probably even more in eastern Trinidad where the climate is wetter, it is only one of several factors and in some years not an important one. Thus for *Manacus,* though under certain conditions rainfall is a proximate factor, as mentioned above, breeding usually begins before the wet season starts and its time of onset is probably determined in part by the varying state of the food supply, which increases in abundance during the first four months of the year; and it may even be affected by the availability of the material used for the lining of the nest (Snow, 1962a).

Day-length

There is still no general agreement as to the role of day-length as a proximate factor controlling breeding seasons in the tropics, where seasonal changes are very small; its importance in temperate latitudes has been amply proved. Recent experimental work has shown that the gonads of tropical and even equatorial birds, which in nature experience no change of day-length, may be stimulated by increasing light (*e.g.,* Marshall & Disney, 1956; Miller, 1959). Uncertainty remains as to how far towards the Equator the diminishing seasonal changes of day-length continue to have an effect, and as to

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[2] Discussion of fresh-water and sea birds is beyond the scope of this paper, but it may be pointed out that the breeding seasons of herons and egrets, ibises, terns, and pelecaniform birds in Trinidad and Tobago are also at their height at the same time, although their ecological requirements are diverse and quite different from those of the land birds. The degree to which their breeding seasons are adjusted to environmental conditions would repay study.

their importance relative to other environmental factors.

Baker (1939), investigating the breeding seasons of a large number of Old World birds from all latitudes, found that April and May, the months of the northern spring, are still the favorite months for egg-laying at latitudes 0-10° N. Correspondingly, at latitudes 0-10° S., September is the favorite month. He suggested that the main proximate factors stimulating breeding in the tropics are, not day-length, but increased light intensity, associated with the overhead sun, and/or wet weather, while length of day and temperature are the most important proximate factors in temperate and boreal latitudes.

Moreau *et al.* (1947), investigating the annual cycles of three species at Amani, 5° S. in East Africa, found that their laying seasons were all more or less the same, October-January. The three species are ecologically so different that they could suggest no reason why it should be most advantageous for all of them to breed in the same months, which, as they pointed out, are "the central breeding months of the Amani bird fauna as a whole, including such dissimilar species as hornbills, rock-martins and bulbuls, and moreover, generally speaking, those of species at higher latitudes throughout the southern hemisphere."

Later Moreau (1950) pointed out that in Africa the breeding seasons begin in general with increasing day-length, but that this is also the time of increasing warmth, onset of the wet season and recrudescence of the vegetation, as it is also at 10° N. in Trinidad. Clearly, broad regional surveys of breeding seasons are of limited value in elucidating the role of individual proximate factors which are thus causally linked together in the main march of the seasons.

In more recent discussions, dealing more specifically with the neotropical region, Miller (1959 and 1960) has suggested that day-length is a significant proximate factor at 10° N. in Central America, and even further south. Miller concludes: "Thus at latitude 10° photoperiodic influences are clearly felt and probably these prevail weakly even to latitude 5°. Certainly they are far from absent at 8° in Panamá." However, Miller bases his conclusion simply on the fact that the peak of the breeding season comes shortly after the spring equinox, when day-length is increasing most rapidly, and does not seem to take full account of the environmental changes associated with increasing day-length, which, as in Trinidad and in the area studied by Moreau, include the onset of wet weather.

TABLE XVII. MONTH OF START OF BREEDING OF SIXTY-TWO SPECIES

(Only those species are included which have a limited breeding season that is known sufficiently exactly)

Month	Number of species starting to breed
January	10
February	7
March	8
April	10
May	3
June	5
July-August	—
September	1
October	5
November	6
December	7

Our data lead to the conclusion that day-length is of little importance as a proximate factor initiating breeding seasons in Trinidad. Table XVII gives an analysis of the months of start of breeding in those species whose breeding seasons were known sufficiently accurately. Nearly one-third of them begin to breed when day-length is decreasing, and only just over one-third in the months (March-May) when it is increasing fastest.

It is thus clear that, whatever the role of day-length, it can have no very general effect in initiating breeding, or else changes in day-length must affect different species, including closely related species, in very different ways. If increasing day-length is responsible for the main peak of breeding, in April-June, as Miller concludes for Central America, its effect on most species must be to cause an increased amount of nesting in the course of a breeding season which has already begun, in some cases months earlier. One of the ways in which it would have to exercise this effect would be by causing re-nesting to follow more rapidly after the end of a previous nesting attempt, as in *Thraupis palmarum* (p. 19) and other species. Conversely, decreasing day-length might be one of the factors responsible for the "winter" decline in nesting which is usual in species with very long breeding seasons. We have no means of testing these hypotheses with the data at hand.

Temperature

Another physical factor which varies seasonally and more or less regularly is temperature. Again, as for day-length, it is unlikely that it can have any general effect in initiating breeding seasons, yet it is possible that, like decreasing day-length, it may play a part in the "winter" decline in breeding. It has already been shown that this decline, which leads to a low level of breeding in the first half of the dry season, cannot be a direct effect of dry weather, as it begins in November or December, while the weather is still very wet. But these are the months when temperatures, and especially the nightly minima, decline quite sharply, and they continue low until March, when they rise equally sharply (Text-fig. 3). It seems reasonable that this period of low minimum temperatures might have the same depressing effect on breeding activities as cold weather in the north.

It must be admitted that the little data that we have to test this suggestion do not lend it much support. Temperatures in the period November-February varied considerably in the three years 1958-59, 1959-60 and 1960-61. The breeding of *Turdus fumigatus* and *T. albicollis,* the only species with long breeding seasons of which enough nests were found for analysis, also varied in the same periods, but not in apparent relationship to the temperatures.

Internal Factors

In species which breed practically throughout the year, the beginning of breeding is probably determined internally, with the recrudescence of the gonads after the moult. Thus *Turdus fumigatus* starts to breed as soon as it has finished moulting and continues until its next moult starts. For such species the role of proximate factors, insofar as they affect breeding, must be limited to inhibiting and re-stimulating breeding within a more or less continuous season.

Most species wait a certain length of time after the end of the moult before beginning to breed. It has already been sufficiently stressed that it is hard to identify proximate factors stimulating breeding in such species, yet they tend to have definite and well synchronized breeding seasons. In the light of Marshall's recent work on the adaptive nature of differences in post-breeding refractory periods in the gonad cycles of different species of birds (Marshall, 1960), it seems likely that the length of time which elapses between the end of the moult and the beginning of breeding depends primarily on specific refractory periods, and is modified by environmental factors which sometimes may be obvious (time of onset of wet season) but more often are too subtle to be apparent to the observer.

THE CONTROL OF THE ANNUAL CYCLE

The arguments outlined in the previous section indicate that the proximate factors affecting the onset and course of the breeding season are unlikely to be of primary importance in controlling the whole annual cycle. In the first place there are those species, already discussed, which breed more or less continuously except when moulting, and moult at the same time each year. Secondly, there are species with restricted and annually variable breeding seasons, which nevertheless moult at the same time each year (*e.g., Manacus, Pipromorpha).* There is a further consideration which is not conclusive but at least highly suggestive, that the breeding seasons of different species start in all months from October to May, or even later, yet practically all of them moult in the same months of the year.

All this suggests that the maintenance of the annual cycle from year to year must depend on the birds' response to some regularly recurring environmental factor or factors whose effect is to induce the onset of moult. There is another important consideration which points the same way. In those species with long breeding seasons—and they are the majority—young birds may be born in many different months of the year; yet in nearly all the species for which we have data the first full moult, which takes place in their second year, is synchronous or nearly so with the post-breeding moult of the adults. There is good evidence that this first complete moult, which thus brings the young bird into synchrony with the annual cycle of the adults, precedes breeding; certainly it does so in the males of manakins, tanagers and honeycreepers, which have distinct juvenile and adult plumages.

In north-temperate species there is experimental evidence that decreasing day-length may induce moult, but day-length is an unlikely controlling factor in Trinidad, as in most species the moult begins at a time when it is hardly altering at all, and in many species the early individuals begin to moult before and the later ones after the summer solstice.

In discussing the annual cycle of *Manacus* (Snow, 1962a), it was suggested that the beginning of the wet season may play some part in determining the time of moult. This suggestion was supported by the data on the annual differences in the time of onset of moult of banded individuals which were followed over two years or more. In 13 out of 17 cases the annual differences in the time of moult were in agreement with the differences in the time of onset of the wet season. On the present data we cannot go further than this; but it may be pointed out that the beginning of the wet season is the most marked seasonal change of the whole year, and is also fairly consistent in its timing from year to year, so that it is perhaps the most suitable environmental time-giver available.

It is unfortunate that our trapping was not continued long enough to provide data for a critical test of the effect of the wet season on the onset of moult in several species. A large-scale trapping program, continued over several years, could provide the necessary data, and would perhaps be more valuable at this stage than the accumulation of more and more breeding records.

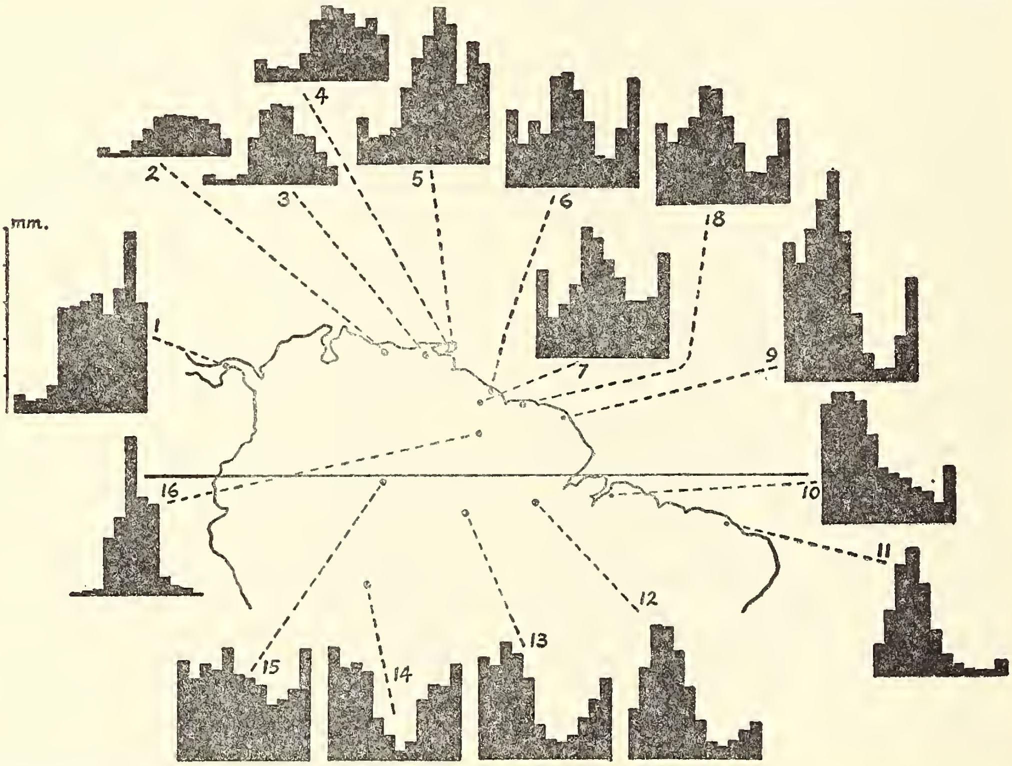
TRINIDAD IN RELATION TO NORTHERN SOUTH AMERICA

The Trinidad Climate in a Wider Context

The climate of Trinidad is broadly similar to that of northern Venezuela immediately to the west—a long dry season, from January to May, is followed by wet weather for most of the rest of the year. This regime is characteristic of northern South America east of the Andes and of much of southern Central America. (We shall not be concerned here with South America west of the Andes, where different climatic conditions prevail).

South of the Equator, at equivalent latitudes, the seasons are almost exactly reversed, or more accurately, shifted by six months—July to November are the driest months of the year, and January to June the wettest. This is the climate of the whole of eastern Brazil. From the present point of view, it is most instructive to consider the change-over from the south-tropical to the north-tropical rainfall regime as one proceeds northwestwards from the corner of Brazil along the coast of northern South America in the direction of Trinidad (Text-fig. 10).

The south-tropical regime remains unchanged as far as Belem, only 1° south of the Equator, except that the wet season begins slightly earlier, in December. The beginning of the change is seen at Cayenne, 4° N.; here the dry season in the second half of the year is restricted, and the wet season is beginning to be broken by a second dry season in February-March. At Paramaribo, in Surinam, the second dry season is more pronounced, and in northern British Guiana it is almost equal to the original dry season in extent, though not in severity. Northwestwards from British Guiana the dry season in the second half of the year rapidly diminishes and the February-



TEXT-FIG. 10. The mean monthly rainfall at various localities in northern South America (all to the same scale, as shown for no. 1). Localities: 1, Barro Colorado, Panama. 2, Caracas, Venezuela. 3, Cantaura, Venezuela. 4, Port-of-Spain, Trinidad. 5, Arima Valley, Trinidad. 6, Georgetown, British Guiana. 7, Mazaruni, British Guiana. 8, Paramaribo, Surinam. 9, Cayenne. 10, Belem, Brazil. 11, Fortaleza, Brazil. 12, Taperinha, Brazil. 13, Manaos, Brazil. 14, Senna Madureira, Brazil. 15, San Gabriel, Brazil. 16, Lethem, British Guiana.

March dry season increases in extent and severity, until the rainfall regime shows the complete six-month shift from that prevailing in northeast Brazil. In Trinidad, a remnant of the July-November dry season still persists, as the "petit carême" already mentioned, being most apparent in the wetter eastern parts of the island. The rainfall of Port-of-Spain, near the northwest corner, is almost identical with that of adjacent northeast Venezuela and shows little evidence of the second dry season.

Inland in northern South America, the rainfall regimes characteristic of the coastal belt are much modified. The July-November dry season extends to much of the lower Amazon basin, but further inland it shifts to earlier in the year, until at Senna Madurera (9° 8′ S., 68° 40′ W.) the driest weather comes exactly in the middle of the year. Some areas are so wet that no dry season is apparent. The other main inland region, the savannas of central Venezuela and southern Guiana, has exactly the opposite rainfall regime. Here the northern dry season prevails, but its beginning comes earlier as one proceeds inland, so that at Lethem, for example, at 3° 18′ N., 59° 46′ W., wet weather prevails from April or May until August, the rest of the year being dry (Gilliard, 1962).

The rainfall regime of much of Central America, as already mentioned, is similar to that of Trinidad and northern Venezuela, with a wet season beginning typically in May and lasting for most of the rest of the year, but falling off in the last three months. As one proceeds further from the Equator and changes in temperature become more marked, this becomes a regime of dry winters and summer and autumn rainfall, such as is characteristic of most of Mexico.

To summarize, the climatic "Equator," at which the north- and south-tropical dry seasons are equally balanced, passes through the extreme north of British Guiana. Trinidad stands only 3 degrees north of it, at the point where the last trace of the south-tropical rainfall regime disappears, being obliterated by the north-tropical regime which from here to the west and northwest prevails over a huge area of northwest South America and Central America. As far as they are ultimately controlled by the wet and dry seasons, we should therefore expect the breeding seasons of Trinidad birds to resemble those of Venezuela and Central America, but perhaps still to be influenced by the double wet and double dry season regime which prevails only a short distance to the southeast.

The discrepancy between the geographical and what may be called the climatic Equator is very important in elucidating the factors controlling the annual cycle, already discussed with reference to Trinidad alone. For if day-length were the main proximate controlling factor, we should expect the half-way point in the shift from the northern hemisphere to the southern hemisphere breeding seasons to occur at the geographical Equator. If, on the other hand, rainfall exerts over-riding ultimate control, we should expect the shift to occur at the climatic Equator. If both exert an effect, we should expect some kind of intermediate condition. It will be seen from the following sections that the effect exerted by the rainfall regime is paramount, while that exerted by changes in day-length is open to question and in any case of minor importance.

The Breeding Seasons in Neighboring Areas

We have quantitative information, of varying fullness, on the breeding season in the following areas: Belem, northern Brazil (Pinto, 1953), and Cantagallo, eastern Brazil (Euler, 1867); northern British Guiana (Davis, 1953; also summarizing Beebe, 1925); northeast Venezuela (Friedmann & Smith, 1955); Costa Rica (Skutch, 1950). In addition, Haverschmidt has published details for several species in Surinam. (The extensive data given by Penard & Penard (1908-10) for Surinam are unreliable (Haverschmidt, 1949)). It will be most convenient to consider these areas in a logical rather than geographical order.

Northeast Venezuela. Friedmann & Smith's data, from an area of savanna and seasonal woodland only about 100 miles from Trinidad, show a sharp rise in breeding activity in March, coming to a peak from April to July, followed by a general decline from August to December which is interrupted by a minor resurgence in October (Text-fig. 11b). The peak is at the same time as in Trinidad, the breeding seasons of the main ecological groups are similar, and those species for which there are data from both areas show good general agreement. The main difference, as would be expected from the difference in climate, is that the breeding season appears to be shorter than in the Arima Valley; possibly it is no shorter than in the northwest corner of Trinidad. There is only one puzzling discrepancy between the northeastern Venezuelan and Trinidad records: *Amazilia fimbriata,* the only hummingbird for which Friedmann & Smith had many records, was found to breed from August to January, a season completely at varianee with that of any of the Trinidad hummingbirds.

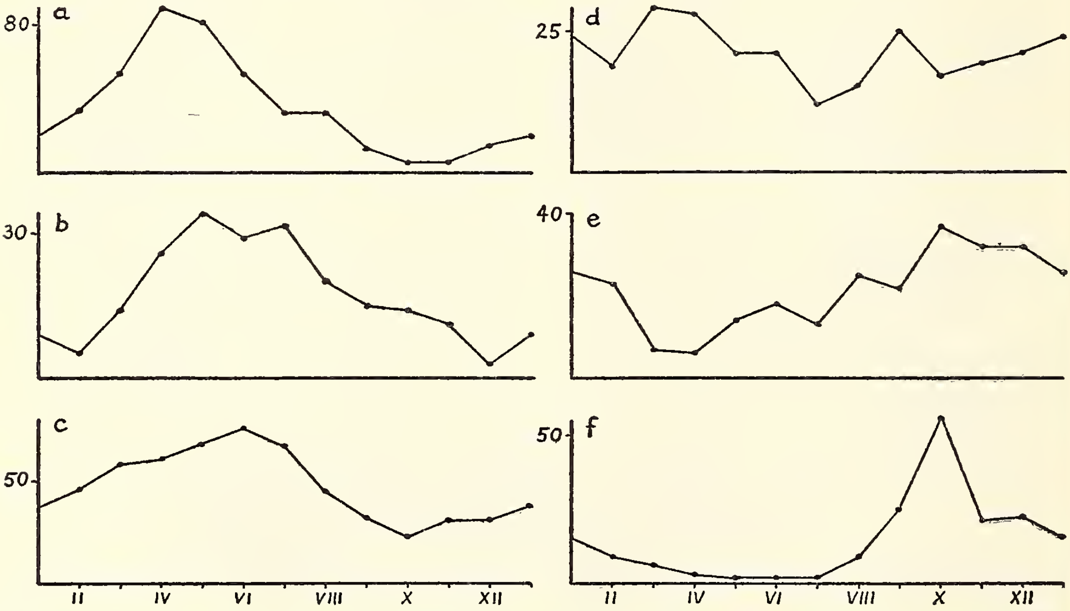
Central America. Skutch's extensive records, based on many years' residence, afford a far more detailed picture of the breeding seasons in

Central America than we have for the other areas reviewed here.

In general, there is a well-marked breeding season with its peak in the months April-June (Skutch, 1950, 1954, 1960). In the valley of El General in southern Costa Rica, where most of Skutch's records were obtained, the amount of breeding gradually increases through January, February and March, the driest months of the year, and reaches its maximum in April, just before the beginning of the wet season. Activity falls off from June to August, and in the last four months of the year there is very little breeding (Text-fig. 11a). A steady rise in breeding activity in the first four months of the year was found at a number of other localities and is doubtless general in Central America. Some groups of birds with specialized feeding habits breed at a different time from the majority, notably the nectar-drinkers and seed-eaters, and in each case Skutch was able to show a correlation with the season when their food was at its most abundant. He also gave convincing evidence that the time when most of the less specialized species were feeding their young (May-June) coincided with the time when the supply of insects and ripe fruit is at its height.

At low altitudes in Central America, the main peak of the breeding season and the associated environmental changes correspond closely with the situation in Trinidad (at high altitudes, as would be expected, the breeding season is more restricted). The chief difference seems to be in the seasonal abundance of flowers and, in association with this, the breeding season of the nectar-drinking birds. In the valley of El General flowers are most abundant in December and January, and least abundant towards the end of the dry season, in late March. Correspondingly, the hummingbirds and *Coereba* breed less in the second half of the dry season than at any other time, whereas in Trinidad the whole of the dry season is a period of abundant flowering and the nectar-eaters breed throughout it. At higher altitudes in Central America, also in agreement with the flowering season, Skutch found an even greater divergence in the breeding seasons of the nectar-drinkers from the general pattern. Thus in the Sierra de Tecpán, at 8-10,000 feet, their breeding was at its height in November and December.

It is noteworthy that, although the climate of the valley of El General is as wet as any part of Trinidad and it is a little nearer the Equator, the main peak of breeding is more sharply defined than in Trinidad, and there is no indication of a secondary peak in October or November. The difference is also apparent if the breeding seasons of species common to both areas are compared (Table XVIII). The climate of El General, for orographic reasons, is much wetter than that of neighboring areas (Skutch, 1950). It



Text-fig. 11. The breeding seasons in central and northern South America (number of species found breeding in each month). (a) Costa Rica (Skutch, 1950). (b) Northeast Venezuela (Friedmann & Smith, 1955). (c) Trinidad. (d) Northern British Guiana (Davis, 1953). (e) Belem (Pinto, 1953). (f) Cantagallo (Euler, 1867).

TABLE XVIII. BREEDING SEASONS OF FOUR SPECIES IN COSTA RICA AND TRINIDAD

	Total nests	% of clutches started in different months											
		Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Tyrannus melancholicus													
Trinidad	33	3	6	9	21	18	12	21	9	—	—	—	—
Costa Rica	—	—	—	x	x	x	x	—	—	—	—	—	—
Elaenia flavogaster													
Trinidad	39	—	3	3	33	36	18	3	—	—	—	3	3
Costa Rica	44	—	—	9	41	32	11	5	2	—	—	—	—
Pipromorpha oleaginea													
Trinidad	61	—	—	3	11	30	36	15	3	2	—	—	—
Costa Rica	16	—	—	25	44	13	13	6	—	—	—	—	—
Thraupis virens													
Trinidad	111	7	4	9	28	19	18	10	1	—	3	1	1
Costa Rica	49	—	—	27	47	20	4	2	—	—	—	—	—

is tempting to suppose that the breeding season is adapted primarily to the drier conditions that prevail over the greater part of the Pacific slopes of Costa Rica, as discussed more fully later (p. 32).

Belem. In the neighborhood of Belem, only 1° south of the Equator, where the wet and dry seasons are almost exactly at the opposite time of the year from the seasons in Trinidad and northeast Venezuela, the breeding season is correspondingly shifted. Incomplete though Pinto's data are, they show that the period of greatest activity is from October to December, the end of the southern spring, and the period of lowest activity is in March and April (Text-fig. 11e). The peak of the breeding season coincides with the time when breeding is at its height at Cantagallo, 22° S., in the neighborhood of Rio de Janeiro (Euler, 1867; Text-fig. 11f), and presumably in all subtropical and temperate parts of South America south of the Equator. Pinto's data further show that the switch-over applies to individual families whose breeding seasons differ from the general pattern. Thus the hummingbirds breed mainly from June to December, *Coereba* from April to December, the tanagers from September to February, and the seed-eaters mainly in the first six-months of the year. It seems almost certain that the main season of moult must be in the months March-July.

British Guiana. Lying at the point where the north-tropical and south-tropical dry seasons are equally balanced, the breeding seasons in British Guiana must be of particular interest. It is therefore unfortunate that, as Davis (1954) points out, his own records differ considerably from Beebe's from essentially the same area. Since Davis's records are based on a longer period of continuous residence, and (like the rest of those used in the present paper) do not include gonad records, which constitute an unknown number of Beebe's records, we have used only Davis's data. These show a considerable amount of breeding in all months, with two peaks, a large one in March and April and a smaller one in September (Text-fig. 11d), the former coinciding with the end of the short dry season and the latter with the beginning of the long dry season. It appears that, although climatically the south-tropical dry season is somewhat better developed than the north-tropical dry season, the general breeding season approximates more closely to the northern type. It would be extremely interesting to have quantitative information on moult in this area. One would especially like to know whether, as would be expected, different individuals of the same species are found moulting at two different times of year, and if so, whether some individuals consistently follow a "northern" and some a "southern" annual cycle.

The data are insufficient to allow analysis of the breeding seasons of many individual families, but the following points may be mentioned. The hummingbirds breed mainly from June to November, at the same time of year as at Belem. Thus the main part of their breeding season coincides with the long dry season, when flowers are most abundant (Davis, 1954). The flycatchers show two distinct peaks coincident with the two general peaks of breeding; though the second peak, in August-October, is of shorter duration than the main January-May peak, the number of species found breeding in the two periods was almost the same. The nightjars, which in Trinidad show a single peak in March-May, breed in both the peak periods in British Guiana, though probably less during the second period than the first.

Surinam. Haverschmidt's data are a valuable supplement to the British Guiana records, as they are much more complete for the few species with which they deal (Haverschmidt, 1950, 1952a, 1952b, 1953, 1954, 1955, 1959). They suggest that very long or continuous breeding seasons are rather common (five out of the seven species: *Columbigallina talpacoti, Tapera naevia, Amazilia fimbriata, Troglodytes musculus, Thraupis virens).* The seasonal distribution of the nests of *A. fimbriata* is closer to that of hummingbirds in Trinidad than at Belem (contrary to Davis's records); the breeding season of *T. leucomelas* is also closer to the Trinidad season for *Turdus* species; but *Chelidoptera tenebrosa,* a member of a family that does not occur in Trinidad, breeds mainly in the second half of the year, and thus follows the Belem breeding season more closely. Data are available for too few species to allow a general discussion, but these records illustrate the transitional nature of the breeding season in the neighborhood of the climatic Equator.

More complete information from the above areas and from other critical areas, associated with moult data, would be necessary before a firm conclusion can be reached, but the data summarized above suggest the following tentative conclusions. At Belem, where day-length is practically constant, there is a well-marked breeding season which is clearly related to the rainfall regime in the same sort of way as are the breeding seasons in Trinidad, Venezuela and Central America. Thus the rainfall regime and the associated environmental changes appear to be adequate to exert effective proximate and ultimate control.

In British Guiana, the breeding season again appears to be related primarily to the rainfall regime, here a double one, but the evidence suggests that though the south-tropical (August-November) dry season is more marked than the north-tropical (February-April) dry season, the pattern of the breeding season has a distinctly more "northern" character. If more complete data confirm that this is so, it might suggest that increasing day-length still exerts a proximate effect even at 7° north. However, the difficulties of attributing an important role to day-length in tropical areas where the breeding seasons of different species are very diverse, and mostly rather long, have already been stressed with respect to Trinidad.

A comparison between the data from Trinidad and Costa Rica (Valley of El General) strongly suggests that the breeding seasons in these two areas cannot be satisfactorily explained without reference to the wider regions of which they form a part. The two areas are at approximately the same latitude, both have heavy rainfalls with the dry season at the same time of year, and they have in common many species of birds or closely related pairs of species. Yet while the Trinidad species begin to breed in all months from October onwards to April or May, their Costa Rica counterparts nearly all begin in March or April (a difference which incidentally further reduces the likelihood that day-length is an important proximate factor). Skutch's area, as already mentioned, is a humid enclave surrounded by country subject to a drier, more seasonal climate. It may well be that essentially the same breeding seasons prevail over the more extensive surrounding areas and so are not perfectly adapted to the valley of El General. Likewise, Trinidad's position, only a short distance north of the Guianas, with two nearly equalled balanced dry seasons and two wet seasons, may have resulted in a breeding season more akin to those areas than Trinidad's climate alone would warrant. Skutch's account of seasonal changes in the flora of his area suggests that the whole biotic complex may be more strongly seasonal than in Trinidad. The possibility must not be overlooked that natural selection has led to complexes of closely knit seasonal changes, affecting both animal and plant life, which may prevail over large areas and be adapted to the general climatic conditions of the region but not perfectly adapted to special local conditions.

Skutch's data make it unlikely that the October peak of breeding in Trinidad has any geographical continuity with the autumn recrudescence of sexual activity in resident northern birds. If this were so, some indication of autumn breeding would be expected in Costa Rica. Instead, as suggested above, it is more likely that the October peak in Trinidad is homologous with the September peak in British Guiana, and thus represents the remnant of the main south-tropical peak of breeding.

Summary

An account is given of the annual cycles of Trinidad land-birds based on 4½ years' field work.

The climate of the central part of the Northern Range of Trinidad, where most observations were made, is humid, precipitation averaging nearly 100 inches per year. There is a single dry season, from January to May. The natural

vegetation is forest. Systematic observations on the flora showed that there is much flowering throughout the year, with ill-defined peaks in April and November, while the abundance of fruits showed a marked peak in April-June and a minor peak in November. Insect life appears to be most abundant at the beginning of the wet season.

The breeding and moulting seasons are described for each family. There is good evidence in several cases that differences in breeding season are related to the availability of food. In species with long breeding seasons, there is a strong tendency for the intensity of breeding to be bimodal, with a minor peak in October or November (following the moult), and a main peak in April-June.

The breeding season varies locally in Trinidad, being longest in the wettest areas in the east and probably shortest in the very dry areas in the northwest of the island. The same species of hummingbirds begin breeding earlier in open country than in forest, but no other marked differences in breeding season according to habitat were noted.

In the five seasons of observation, no difference was found in the breeding seasons of some species, while in others annual differences of up to five months were noted.

The number of broods per year and intervals between successive broods were determined for several species. In general, 2-4 nesting attempts per year are usual. Intervals between broods vary according to species; in species with long breeding seasons they tend to be short at the peak of the breeding season (usually around May) and long in the dry season.

The moult occurs mainly from July to November, with little annual variation. The rate of moult and total duration of the moult were ascertained for several species.

The ultimate and proximate factors controlling the breeding season are discussed. Food supply is considered to be the main ultimate factor determining at what season each species breeds. Proximate factors are much more obscure, but for some species, at certain times, rainfall is most important. Reasons are given for supposing that changes in day-length cannot be of general importance.

The relative constancy of the season of moult, and variability of the breeding season, both within species and between different species, as well as other considerations, suggest that the long-term control of the whole annual cycle may depend on environmental factors initiating the moult rather than on factors initiating breeding.

A comparison is made between the breeding seasons in Trinidad and other parts of the neotropical region. The survey emphasizes the importance of the rainfall regime and associated environmental changes in both the ultimate and proximate control of breeding seasons. It is suggested that in limited areas breeding seasons may not be perfectly adapted to local conditions, but rather to the climate and associated seasonal changes prevailing over the wider region of which they form a part.

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APPENDIX

Breeding and Moult Records of Trinidad Land-birds

The following table presents a summary of breeding and moulting data for those species for which records were obtained in the course of the present study. A number of published breeding records are also included which satisfy the requirements given on page 3; the sources of all these are listed at the end of the Appendix.

The figures show the numbers of nests in which the first egg was known or calculated to have been laid in the month indicated. All months in which individuals undergoing wing-moult were trapped are indicated by a dash, either by itself or below the figure if nests were also recorded in the same month.

Species	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Crypturellus soui		1	1	1		1	1	1	1	1	1	
Columbigallina passerina		1				1	1 —	1				
Columbigallina talpacoti	7 —	11 —	14	6	4 —	8 —	17 —	18 —	22 —	4 —	9 —	8 —
Columbigallina minuta			1	2	2	1		2	2			
Leptotila verreauxi	2	3	9	8	6	1	5	—	2	4	5	
Leptotila rufaxilla	1	2	4	4	2	5 —	4 —	1 —	—		1	1 —
Geotrygon montana		1		1	3		1					
Touit batavica	1	3										
Tapera naevia			1		1	4	1	1	3	2		
Piaya cayana	1				1		2				1	
Crotophaga ani	1	8	3 —	4	7	6 —	7 —	5 —	12	8	4 —	1
Otus choliba		1	4	2	2							
Glaucidium brasilianum		1	1	1	2		1					
Steatornis caripensis	12	8 —	20 —	10 —	11 —	2	3	3 —	1	—		9
Nyctibius griseus			2	3			1					
Nyctidromus albicollis					2							
Other nightjars	1	1	3	5	1			1				
Chaetura cinereiventris					1	—		—		—	—	
Chaetura spinicauda						1		—				
Chaetura brachyura				3	30	22	18	11 —	4 —			
Cypseloides rutilus		—		1	6	8	8	9	—			
Panyptila cayennensis			1		1							
Glaucis hirsuta	38	69	46	75	71	58	28	1 —	—	—	—	6
Phaethornis guy	4	5	4	5	3 —	4 —	2 —		—	—	1	2
Phaethornis longuemareus	4	5	2			1	—		—			1
Anthracothorax nigricollis	4	3	4	5	5	2			—		—	
Chrysolampis mosquitus	5	6	5	4	9			1				1
Chlorestes notatus					2	2	—	—	—		—	—
Amazilia chionopectus	2	1	3	1			—	—	—		—	1
Amazilia tobaci	7	7	10	4	5 —	3 —	3 —	1 —	—		1	3
Trogon viridis							1					
Trogon collaris			1									
Trogon violaceus		1	1		1		—				1	
Momotus momota				4	3	—	—			—		
Galbula ruficauda		2	7	7		1 —						
Piculus rubiginosus			1	1	4			—	—			
Celeus elegans				1	3		—	—	—		—	
Veniliornis kirkii	1	1							—			1
Xiphorhynchus guttatus			1			2 —	2 —	—	—			
Dendrocincla fuliginosa					2	4 —	1	1 —	1 —		—	—
Synallaxis albescens	2			1	1	2	3	1	5			2
Synallaxis cinnamomea		1			1				1		3	1

BREEDING AND MOULT RECORDS OF TRINIDAD LAND-BIRDS *(continued)*

Species	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Certhiaxis cinnamomea		—				3	2	1	1	2	—	
Sclerurus albigularis	3	2								1	2	5
Sakesphorus canadensis					2		2				—	
Thamnophilus doliatus	$\underline{3}$	3	—	$\underline{1}$	2	$\underline{3}$	$\underline{2}$	—	$\underline{2}$	$\underline{1}$	$\underline{1}$	$\underline{1}$
Myrmotherula axillaris					—	2	1	1	—			
Formicivora grisea							1	1				
Myrmeciza longipes									1			1
Formicarius analis			2						1			
Pachyrhamphus polychopterus			$\underline{1}$	1		1	1		1			
Tityra cayana		1	1								1	
Procnias averano				1	2		1	1		1	1	
Pipra erythrocephala	1		$\underline{2}$			4	$\underline{5}$	$\underline{1}$	—	—	—	—
Manacus manacus	$\underline{9}$	23	19	27	46	$\underline{69}$	$\underline{37}$	$\underline{35}$	$\underline{6}$	—	—	$\underline{1}$
Fluvicola pica	—					$\underline{3}$	1	3	1	1	—	—
Arundinicola leucocephala			1				1	3	2	1		
Tyrannus melancholicus	1	2	3	7	6	4	7	3				
Legatus leucophaius		1	2	1	3	3						
Myiodynastes maculatus			1			2						
Megarynchus pitangua		1	1	4	5	2						
Pitangus sulphuratus	3	4	2	7	7	3	1	$\underline{2}$		1	$\underline{1}$	2
Contopus cinereus			2		3	1	3		—			
Empidonax euleri						1		—		—		
Myiophobus fasciatus			—	1	1		3	3		—	1	—
Platyrinchus mystaceus					1							
Tolmomyias sulphurescens				1		3	2					
Tolmomyias flaviventris							1	1	1			
Elaenia flavogaster		1	1	13	14	7	1	—		—	$\underline{1}$	1
Myiopagis gaimardii		1										
Camptostoma obsoletum				1	—		1			1	1	
Leptopogon superciliaris	—		1		4	1	1	—				—
Pipromorpha oleaginea			2	7	$\underline{18}$	$\underline{22}$	$\underline{9}$	$\underline{2}$	$\underline{1}$	—		
Progne chalybea				1	7	4	2					
Stelgidopteryx ruficollis				1	3			—				
Thryothorus rutilus	4	1	$\underline{2}$	4		3	3	—				
Troglodytes musculus	5	5	2	8	11	10	5	$\underline{4}$	7	13	7	8
Mimus gilvus	8	9	10	8	13	5	2		4	4	1	
Turdus albicollis	1	6	10	9	11	$\underline{20}$	3	3			7	4
Turdus nudigenis				2	52	53	33	$\underline{11}$	—	—	—	
Turdus fumigatus	$\underline{8}$	19	18	23	26	36	$\underline{19}$	$\underline{1}$	—	$\underline{2}$	18	11
Platycichla flavipes			1	1	1	1						
Ramphocaenus melanurus		1		1		1		3				—
Cyclarhis gujanensis						$\underline{1}$	3	—	1	—	1	
Vireo olivaceus				1	1	3		—				
Hylophilus aurantiifrons							2		—	1		
Chlorophanes spiza		—		—	1	1	1		—			
Cyanerpes cyaneus			1			1	1					
Cyanerpes caeruleus				2		1		—	—	—	—	
Dacnis cayana			2			$\underline{2}$	$\underline{2}$	—	—			
Coereba flaveola	23	7	15	9	$\underline{6}$	$\underline{2}$	—	$\underline{1}$	—	—	1	2

Breeding and Moult Records of Trinidad Land-birds *(continued)*

Species	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Conirostrum bicolor			1			1						
Geothlypis aequinoctialis		1			1			1		1		
Basileuterus culicivorus			1	1	2	1						
Psarocolius decumanus	+	+	+	+	+							
Cacicus cela	9	21		11	6							1
Psomocolax oryzivorus	2	3			1							
Molothrus bonariensis					2	1	—	2	1	1		
Quiscalus lugubris	1	1			3	10	10	2			1	3
Icterus chrysocephalus		1										
Icterus nigrogularis	1		1	4	4	4	1	1				
Agelaius icterocephalus	—					1	1		3	5	3	
Tanagra trinitatis			2									
Tanagra violacea	1		1	2	5	5	5	1				
Tangara chrysophrys	2			—		1		—				
Tangara mexicana				2	1	2	2	1	—	1		
Tangara gyrola	1		1	1	—	—	3	1	—			
Thraupis virens	8	4	10	31	21	20	11	1		3	1	1
Thraupis palmarum	3	4	21	20	15	25	16	12	1	3	1	1
Ramphocelus carbo	33	14	14	19	20	35	14	9	1	—	—	3
Piranga flava							1			1		
Habia rubica		1	1		3	11	3	2	—	1		
Tachyphonus rufus		1	1	2	6	7	2	1	—	—	1	
Tachyphonus luctuosus				1	1				—	—	—	
Saltator caerulescens	—		2	4	10	3	5	1	1	—	—	—
Tiaris fuliginosa	1				1	—		1	1	2	2	2
Sporophila intermedia	—					3	1	4	1		—	—
Sporophila nigricollis							3				2	
Sporophila lineola						1	5	6				
Sporophila minuta		—			1	2	4	—				1
Oryzoborus angolensis						1			1			
Volatinia jacarina	4	—			2	7	10	8	1	—	1	1
Sicalis flaveola									1			

Sources of Records, Other Than Those Obtained in This Study

(The following abbreviations have been used for authors: B. & S., for Belcher & Smooker (1934-37); J. & M., for Junge & Mees (1958).)

C. soui Williams (1922), Oct.
C. passerina: Williams (1922), Feb., Aug.
L. verreauxi: J. & M., Nov. (1).
L. rufaxilla: Chapman (1894), Feb. (1); Roberts (1934), Aug. (1).
G. montana: B. & S., May (3).
T. batavica: B. & S., Feb. (2).
P. cayana: B. & S., July (2).
T. naevia: Roberts (1934), Aug.; B. & S., June (1).
C. ani: Williams (1922), Feb., July, Aug., Sep. (1 each).
O. choliba: B. & S., Mar. (1), Apr. (1); Chenery (1956), May (1).
G. brasilianum: Williams (1922), May (1); B. & S., Feb., Mar., May (1), July.
N. griseus: Muir (1925), Mar. (1), Apr. (1); B. & S., Mar., Apr., July (1 each).
N. albicollis: Williams (1922), May (1).
P. cayennensis: B. & S., Mar.
P. guy: Williams (1922), Dec. (1); J. & M., Jan. (1).
P. longuemareus: Williams (1922), Jan. (3), Dec. (1); B. & S., Mar. (1).
C. mosquitus: J. & M., Dec.
A. chionopectus: Chapman (1894), Mar. (1); B. & S., Mar. (1).
T. viridis: B. & S., July.
M. momota: B. & S., Apr. (1), May (1).
P. rubiginosus: B. & S., May (2).

C. elegans: B. & S., May (2).
V. kirkii: B. & S., Feb.
X. guttatus: B. & S., Mar., June (1).
D. fuliginosa: B. & S., June (2).
S. albescens: Williams (1922), Jan. (1), June (2), Aug., Sep. (4); B. & S., July (2), Dec. (1).
S. cinnamomea: B. & S., Sep., Nov. (1); J. & M., Nov. (1), Dec.
S. cinnamomea: Roberts (1934), Aug.
S. albigularis: B. & S., Oct.; J. & M., Jan. (3), Nov. (1), Dec. (3).
S. canadensis: B. & S., May (1), July (2).
T. doliatus: B. & S., Jan. (1), Feb. (1), June (2), July (1), Dec.
M. axillaris: B. & S., June (1).
F. grisea: B. & S., June.
F. analis: B. & S., Mar. (1); J. & M., Sep.
P. polychopterus: B. & S., Apr., June, July, Sep.
P. averano: Beebe (1954), May (1).
P. erythrocephala: B. & S., Jan., Mar. (1).
F. pica: Williams (1922), June (2), Aug. (3), Sep., Oct.
A. leucocephala: Williams (1922), July, Aug. (1), Sep. (1); Roberts (1934), Aug. (1).
T. melancholicus: Williams (1922), June (1); J. & M., July (1). Aug. (1).
P. leucophaius: B. & S., Apr. (1).
M. maculatus: B. & S., June (2).
M. pitangua: B. & S., Mar., May (2); Street (1946), May (1).
P. sulphuratus: Williams (1922), Apr. (1), June (1).
C. cinereus: Street (1946), May (1).
M. fasciatus: J. & M., July (3), Aug. (3), Nov.
C. obsoletum: Roberts (1934), July.
P. chalybea: B. & S., Apr., May (1).
T. musculus: Williams (1922), Aug. (1), Sep. (1), Nov. (2), Dec. (1); Roberts (1934), June (1); J. & M., Oct. (1).
P. flavipes: B. & S., Apr., May, June.
R. melanurus: B. & S., Apr.; J. & M., Aug. (1).
H. aurantiifrons: Roberts (1934), July (1).
C. flaveola: Williams (1922), Nov., Dec. (1).
G. aequinoctialis: B. & S., May, Aug., Oct.
B. culicivorus: Cherrie (1908), Mar.
P. oryzivorus: B. & S., Jan. (1), Feb. (2).
M. bonariensis: B. & S., Sep.
Q. lugubris: Williams (1922), June (1), Aug. (1).
I. nigrogularis: Williams (1922), May (1), June (1), July, Aug.
A. icterocephalus: Williams (1922), July.
T. trinitatis: B. & S., Mar. (1).
T. mexicana: B. & S., May.
T. palmarum: Williams (1922), June, July, Aug., Sep., Dec. (1 each).
T. rufus: Cherrie (1908), Mar.; Williams (1922), June (2).
T. luctuosus: B. & S., Apr., May.
T. fuliginosa: B. & S., Jan., May, Aug., Sep., Oct. (2), Nov. (2), Dec. (2).
S. intermedia: Williams (1922), Aug. (4).
S. minuta: Williams (1922), July (2).
O. angolensis: Williams (1922), Sep.
V. jacarina: Williams (1922), Aug. (2), Nov.

2

The Effect of Thermal Acclimation on Brain Cholinesterase Activity of the Killifish, *Fundulus heteroclitus*[1]

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(Text-figures 1-5)

Introduction

The purpose of this study was to investigate some of the biochemical factors involved in the functional failure of the central nervous system in response to thermal stress in poikilothermic animals. The effect of thermal stress on the enzymatic activity and properties of brain cholinesterase (ChE) from the killifish, *Fundulus heteroclitus,* after a period of acclimation to high and low temperatures, has been investigated and an attempt has been made to correlate some of these findings with physiological and electrophysiological data that have been reported previously.

The studies on temperature acclimation in fish reviewed by Brett (1956) and Fisher (1958) indicate that acclimation to thermal stress occurs on the organism, organ, tissue, cellular and biochemical levels. In addition, these reviewers and others (Fry, 1947; and Roots & Prosser, 1962) suggest that the ultimate cause of heat and cold death in fish is intimately associated with a failure of the central nervous system. Since the upper and lower lethal temperature limits for fish vary directly with the acclimation temperature (Brett, 1956), it is clear that some modification occurs in the nervous system in response to thermal stress, which alters its physiological state.

Fries (1952) has observed chill coma in fish and describes a syndrome of numbness, convulsion and pectoral paralysis just preceding death. This entire syndrome, including the striking condition in which the pectoral fins are immobile and stand at a 90° angle to the body, can be duplicated in fish by the action of various anticholinesterases (Weiss, 1961), which suggests that cold death in fish may be due to the physiological limits set by the enzyme ChE of nerve tissue.

The enzymatic activity and some of the properties of fish brain cholinesterases have been investigated (Nachmansohn *et al.,* 1941; Lindeman, 1945; Augustinsson, 1948, 1949; Augustinsson & Fange, 1950; Brightman & Albers, 1959; Baslow & Nigrelli, 1961; Weiss, 1958, 1959, 1961, and Brik & Yakovlev, 1962). The work described in this paper concerns the effect of altered ambient temperature on the activity and some of the properties of fish brain ChE initially, during and after thermal acclimation to high and low temperatures.

Materials and Methods

The animals used in this investigation were essentially marine or brackish water fishes, inhabiting a wide range of temperature zones. Unless otherwise indicated, the northern fishes were adapted to 15° C and the southern fishes to 25° C. All were in prime condition at the time of sacrifice. Fish used for adaptation experiments were maintained on a diet of fish, chopped clams and frozen brine shrimp. The killifish, *Fundulus heteroclitus,* was used as an experimental animal for acclimation studies because of its hardiness, ability to tolerate large temperature variations and availability in large homogeneous populations. The temperatures to which this species was exposed were similar to their normal summer and winter thermal range.

North Atlantic fishes:

Common killifish, *Fundulus heteroclitus;*

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[1] Supported by a grant to the New York Zoological Society by the John A. Hartford Foundation.

northern blackfish, *Tautoga onitis;* common goldfish, *Carassius auratus* (acclimated to 22° C); northern stargazer, *Astroscopus guttatus.*

South Atlantic and South Pacific fishes:

Nassau grouper, *Epinephelus striatus;* Holbrook's porgy, *Diplodus holbrookii;* spot snapper, *Lutianus synagris;* fantail filefish, *Monocanthus spilosoma;* tomato clownfish, *Amphiprion frenatus;* anemone clownfish, *Amphiprion percula.*

Fishes used for acclimation studies were kept in a ten-gallon running sea water system, fed from a ten-gallon reservoir in which the water temperature could be maintained at any temperature between 12 and 35° C, plus or minus one-half degree. The fresh sea water, which came in from a well point at 12° C, was aerated and adjusted to the proper temperature with two thermostatically controlled 250-watt heaters prior to flowing into the experimental tank.

The assay technique used was the colorometric method described by Hestrin (1949), which utilizes a reaction between hydroxylamine and unhydrolyzed acetylcholine. This is the method used by Weiss (1958, 1959) for the determination of fish brain ChE after treatment with various anti-cholinesterases. Fish brains were obtained by removing the top of the skull and cutting the brain loose at the optic nerves and the base of the medulla, after the fish was incapacitated by severing the spinal cord at the level of the pectoral fins. The brains were then washed in cold barbital buffer solution (Lundin, 1959) to remove blood clots, weighed and then homogenized in a ground glass homogenizer in a small amount of buffer (pH 8.1). The resulting brain brei was diluted with additional buffer to 1-4 milligrams of brain tissue per milliliter of solution.

For analysis, one-half milliliter of diluted brain brei was pipetted into a test tube, preincubated for 5 minutes at the assay temperature, and then incubated with one-half milliliter of 0.008 M acetylcholine chloride (Augustinsson, 1949). The standard assay time was 20 minutes at 26° C, plus or minus 0.5° C. Residual substrate was determined by the method of Hestrin (1949) and the enzymatic activity of the brain tissue is reported in terms of milligrams of substrate (acetylcholine chloride) hydrolyzed by the enzyme present in 100 milligrams of tissue per hour. This value is notated Q_{ChE}.

Observations and Results

A. Effects of temperature variation on brain ChE activity *in vitro.*

1. Determination of Q_{10} of ChE. The Q_{10} or factor for increase in enzymatic activity for a 10° C rise in temperature of brain ChE from various species of fish was determined by incubation of enzyme and substrate at different temperatures from 0 to 40° C. It was found that the Q_{10} for the enzyme from each species of fish was fairly constant over a wide temperature range (10-35° C), and thus could be used to determine the specific enzyme activity for any temperature within this range once a standard determination had been made. Below 10° C the Q_{10} of ChE from several fish species increased greatly, thereby causing a significant loss of enzyme activity within a few degrees. It is interesting to note that this occurred in preparations from the filefish and snapper, two "southern" fishes. In a number of "northern" fishes whose brain ChE was analyzed at low temperatures, including the goldfish, blackfish and killifish, the Q_{10} for this enzyme remained fairly constant down to 2° C, at which point the Q_{10} increased and the enzymatic activity decreased sharply as it did in "southern" fishes. The Q_{10} values for brain ChE from killifish acclimated to 12 and 30° C were similar. The possibility that

Table I. Analysis of Various Fish Species for Brain ChE Activity and Enzymatic Q_{10} Values.

Species	Brain weight (mgs)	Total body weight (gms)	Q_{ChE} at 26°C	Q_{10} 15-25°C
Common goldfish	70.3	6.3	41	1.40
Northern blackfish	153.0	60.0	57	1.19
Common killifish	29.7	5.6	48	1.30
Northern stargazer	41.2	58.5	46	1.25
Holbrook's porgy	407.4	221.0	57	1.17
Nassau grouper	895.2	6940.0	28	1.25
Tomato clownfish	32.1	3.8	36	1.40
Anemone clownfish	23.6	2.3	53	1.45
Spot snapper	904.0	937.6	41	1.15
Fantail filefish	113.0	21.0	99	1.02

TABLE II. ANALYSIS OF THE DENATURATION OF BRAIN ChE FROM VARIOUS FISH SPECIES AT ELEVATED TEMPERATURES *in vitro*.

Species	Incubation temperature (°C)	Incubation time (min.)	Loss of ChE activity (%)
NORTH ATLANTIC FISHES			
Common goldfish	26	30	0
	40	30	6
	40	60	12
Northern blackfish	26	120	0
	35	120	5
	40	30	41
Common killifish	26	60	0
	34	60	22
	40	15	50
	40	30	78
	40	60	100
SOUTH ATLANTIC AND SOUTH PACIFIC FISHES			
Tomato clownfish	26	60	0
	35	60	11
	40	30	9
	40	60	21
Anemone clownfish	35	60	7
	40	30	13
	40	60	21
Spot snapper	35	60	0
	40	60	11
Fantail filefish	35	60	0
	40	90	6

there are differences in the properties of ChE from warm and cold living fishes is indicated.

In Table I representative values for enzymatic activity (Q_{ChE}) measured at 26° C and Q_{10} for brain ChE from several species of fish are given. Q_{10} values range from 1.02 to 1.45 and are derived from measurements made at 15 and 25° C.

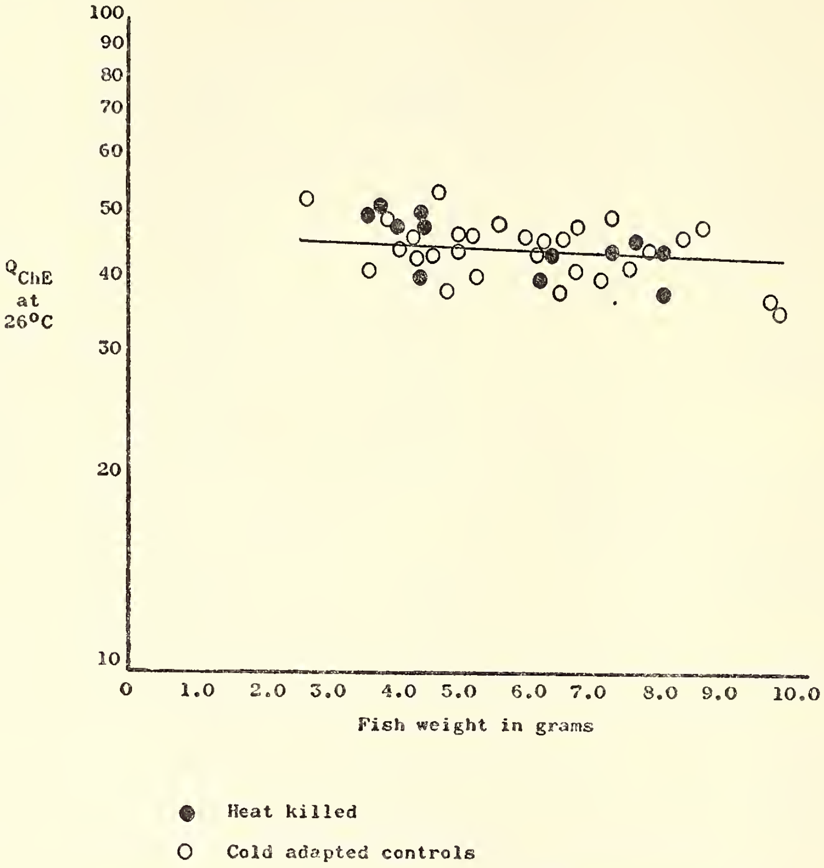
2. Enzyme denaturation at elevated temperatures. At temperatures above 30° C it was found that brain ChE from various sources became rapidly inactivated *in vitro*. In order to determine the degree of denaturation quantitatively, the enzyme preparation was pre-incubated at elevated temperatures for various periods of time, cooled, and then assayed at 26° C with the substrate acetylcholine for residual enzymatic activity. These results are shown in Table II.

Although the number of species involved is small, it is evident that the warm water species exhibit a more heat-stable enzyme than the cold water species, which again indicates basic differences in the properties of brain ChE from cold and warm water forms.

B. Effects of temperature variation on brain ChE *in vivo*.

1. Effect of heat death on brain ChE activity levels of the killifish *Fundulus heteroclitus*. In order to determine if the ChE degradation found to occur at elevated temperatures *in vitro* also occurred *in vivo*, a number of animals were heat killed and their brains analyzed for residual enzymatic activity. The fish were obtained from cold sea water (13° C) and placed in an aerated three-gallon glass aquarium. The temperature was raised to 30° C within 30 minutes, and to 35° C in another 20 minutes. The fish were killed by raising the water temperature to 40° C within the next 16 minutes. The endpoint was reached when the animals floated at the surface and twitched occasionally, but had no control over their swimming movements. After cutting the spinal cord the fish were plunged into an ice water bath and prepared for assay along with a control group of fish. The results of this experiment are seen in Text-fig. 1 and it will be noted that there are no differences between heat killed and control animals.

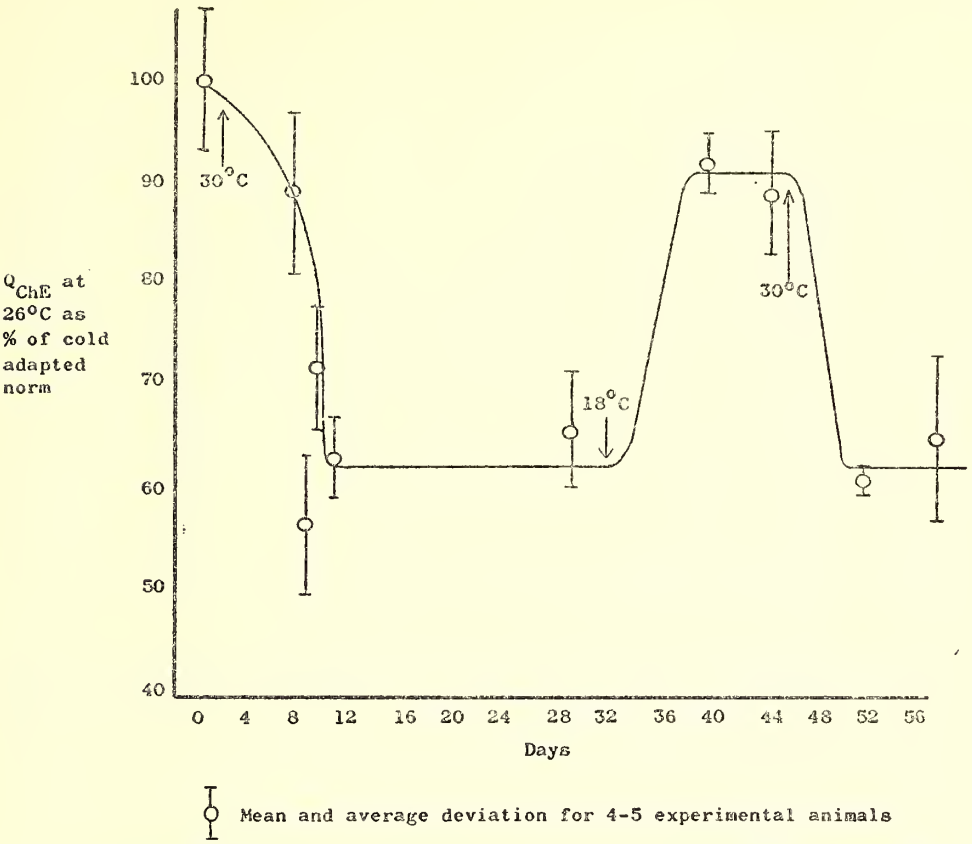
2. Effect of temperature stress on brain ChE activity levels of the killifish, *Fundulus heteroclitus*. To determine the effect of temperature



Text-fig. 1. Brain ChE activity of heat-killed *Fundulus heteroclitus.*

stress and acclimation on the activity of ChE in the central nervous system of the killifish *in vivo,* a large group of animals acclimated to 12° C were placed in a running sea water system capable of maintaining any temperature between 12 and 35° C, plus or minus one-half degree. These fish were then stressed over a period of eight hours by slowly increasing the ambient water temperature to 30° C. Brain samples from 4-5 experimental animals were analyzed for ChE activity on days 7, 8, 9, 10 and 29. After 32 days at this temperature, the population was again subjected to thermal stress by reducing the ambient temperature to 18° C. Determinations of brain ChE activity were made after 7 and 12 days at this temperature. After 13 days at 18° C this group of fish was once more stressed with a temperature of 30° C and samples were taken after 7 and 13 days for analysis of brain ChE activity. The results presented in Text-fig. 2 show that the brain ChE activity of the killifish varies inversely with the ambient temperature, and is approximately 40% lower after acclimation to 30° C than when acclimated to 18° C when the enzymatic activity is measured at 26° C. Non-stressed cold-acclimated killifish were used as controls when determinations of brain ChE activity of the experimental population were made.

A second group of cold-acclimated killifish was stressed with a temperature of 30° C and the changes occurring in brain ChE activity were followed daily, in addition to changes in the opercular rate. The results of this experiment are shown in Text-fig. 3. The reduction in brain ChE activity begins immediately in response to heat stress and is a time-dependent function, being completed in about six days. Opercular rate is also affected immediately upon warming and reduction to the prewarming level is also a time-dependent function which coincides with the reduction in ChE activity. After 7 days, this population was stressed with a temperature of 13° C and it was observed that the animals required about 5 days at the lower temperature before any elevation in ChE activity was evident. Thus, it appears that acclimation on the biochemical level occurs much more rapidly to



TEXT-FIG. 2. Brain ChE activity levels in response to thermal stress in *Fundulus heteroclitus*.

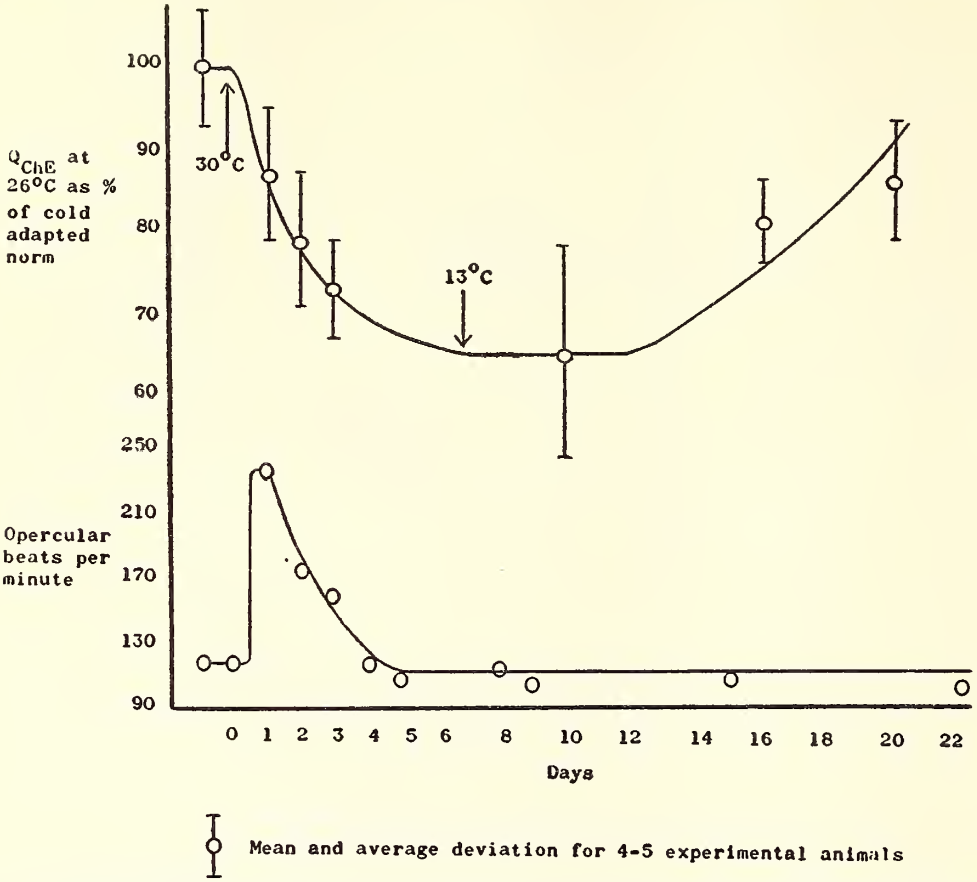
warm than to cold stress. It will also be seen in Text-fig. 4, when enzymatic activity is plotted as a function of the ambient temperature, that the effect of acclimation is to reestablish a specific enzyme activity of approximately $Q_{ChE}=31$ at each new temperature. The specific brain ChE activity of animals adapted to 13° C, when measured at that temperature, is approximately $Q_{ChE}=31$. When stressed with an ambient temperature of 30° C this value goes up to about 50. After a period of acclimation the ChE activity level drops again to about 31 when measured at the new ambient temperature. When the warm adapted animals are cooled to 13° C once again, the brain ChE activity drops to about $Q_{ChE}=20$ initially, but rises to approximately 31 after a period of acclimation to the lowered ambient temperature.

C. Seasonal variation of brain ChE activity of *Fundulus heteroclitus* when maintained at a constant temperature.

When the values for brain ChE of cold water control animals, measured at 26° C, are plotted by month against the October mean value (100%), it is apparent that a rise in brain ChE activity occurs. This rise is almost inperceptible at first, but at the end of January and beginning of February the ChE activity level rises rapidly and reaches a value of more than 130% of the October mean (Text-fig. 5). This variation occurred in a population of fish being maintained in a cold sea water reservoir whose temperature did not vary more than 2° C in five months. Since the population of fish as determined by weight and brain-body proportions did not change during this period, the rise in ChE activity could not be due to selection of smaller fish with higher brain Q_{ChE} activity levels.

DISCUSSION

The results obtained in this investigation (Table I) for brain ChE activity in various teleost fishes are similar to those obtained by Augustinsson (1948) and show that large species differences exist. Even within the limited number of species examined, a three-fold variation in brain ChE levels was found, ranging in enzymatic activity levels from Q_{ChE} 28 to 99. When



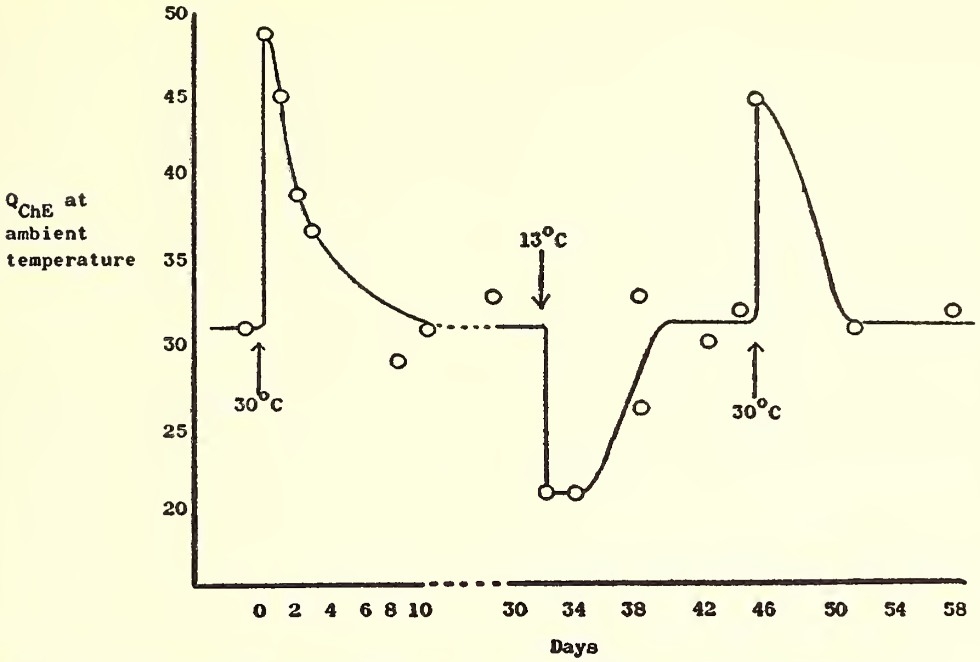
TEXT-FIG. 3. Brain ChE activity levels and opercular rate in response to thermal stress in *Fundulus heteroclitus.*

large populations of a single species are analyzed, however, individual variations are relatively small, usually between 5-10% of the mean ChE level of the species, providing that the body size and thermal history of the sampled population are similar (Text-fig. 1). This has been found for the killifish during this investigation, and has also been reported for the bluegill sunfish, *Lepomis macrochirus;* largemouth bass, *Micropterus salmoides*; golden shiner, *Notemigonus crysoleucas* and common goldfish, *Carassius auratus* (Weiss, 1959). Values for brain ChE activity have been reported for other fish species and the Q_{ChE} activity levels have been found to vary in teleosts from 0.5 to 60 (Nachmansohn *et al.,* 1941; Lindeman, 1945; Augustinsson, 1948; Augustinsson & Fange, 1950; Weiss, 1959; and Baslow & Nigrelli, 1961).

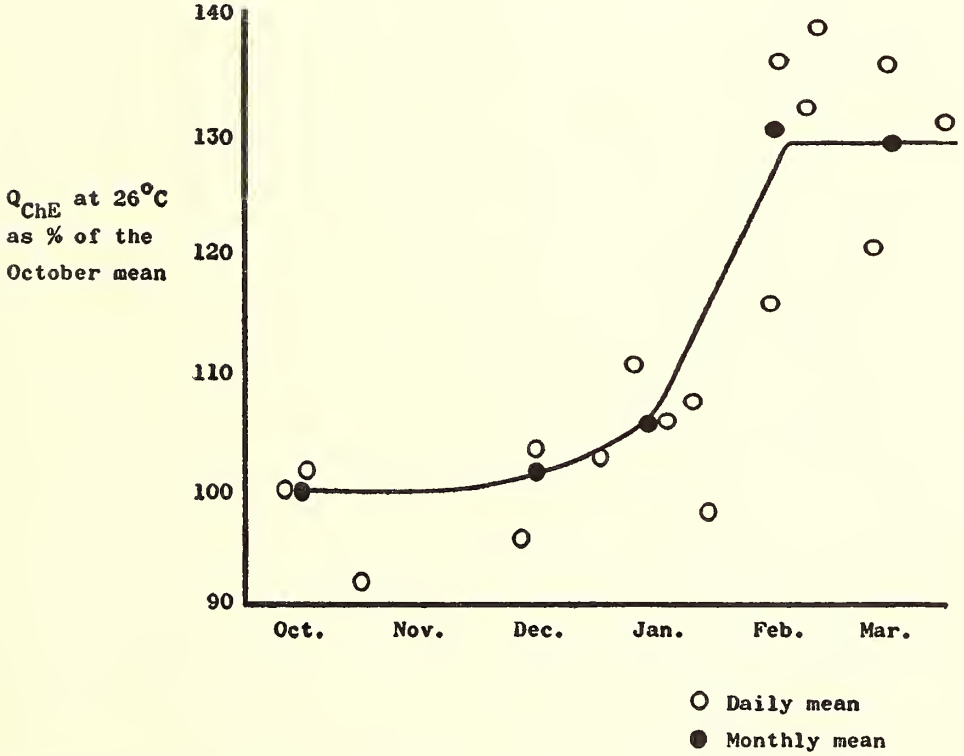
It has also been noted (Text-fig. 1) that as the size of the brain increases in *Fundulus heteroclitus* the ChE activity per unit weight of brain tissue decreases. This has been found to be true for the rat (Roderick, 1960) and also sunfish, largemouth bass, golden shiner, and common goldfish by Weiss (1958, 1959, 1961).

Determinations made over a wide temperature range *in vitro* show that the Q_{10} of brain ChE for several species of fish varies between 1.02 and 1.45 (Table I.) These values are similar to those described by Chadwick (1957) for cholinesterase from various sources but somewhat less than the value of 2 derived for the cholinesterase of the intestine of the catfish, *Ameiurus nebulosus,* by Laurent (1952).

Determinations of residual brain ChE activity after exposure to elevated temperatures have shown that ChE from various fish species is denatured rapidly above 30° C *in vitro* (Table II). This has also been demonstrated for brain oxidative enzymes of the goldfish (Freeman, 1950) and for the enzymes associated with oxidative metabolism of the brain of the largemouth bass by Fuhrman *et al.* (1944). The temperature of inactivation of those enzymes associated with oxidative metabolism *in vitro* corresponds very well, in many cases, with the known upper lethal



TEXT-FIG. 4. Enzymatic activity as a function of ambient temperature in *Fundulus heteroclitus.*



TEXT-FIG. 5. Seasonal variation in brain ChE activity of *Fundulus heteroclitus* when maintained at a constant temperature of 13° C.

temperatures for the respective fish species. In these cases, enzyme inactivation has been suggested as a possible cause of heat death due to exposure to high temperatures. In order to determine whether the brain ChE *in vitro* instability to elevated temperature also occurred *in vivo,* and to see if this instability could be responsible for functional failure of the central nervous system, "heat death" experiments were performed as described previously. The experimental animal *Fundulus heteroclitus,* which exhibited a very heat labile brain ChE *in vitro* (Table II), was exposed to environmental temperatures between 35 and 40° C for more than 20 minutes, or until heat death occurred. *In vitro,* this time and temperature exposure should have resulted in approximately a 30% reduction in brain ChE activity. The results show, however, that no enzyme denaturation had occurred at the time of heat death due to exposure to elevated temperature (Text-fig. 1). It appears, then, that the thermal instability of brain ChE observed *in vitro* does not occur *in vivo* and therefore cannot contribute to functional failure of the central nervous system at elevated temperatures.

It has been noted that the activity of many enzymes in poikilotherms adapts inversely to the ambient temperature when measured at a constant temperature (Precht, 1951; Christophersen & Precht, 1952; Stangenberg, 1955; Kono & Nakagami, 1957; and Kanungo & Prosser, 1959). This has been found to be true also for brain ChE of the killifish. The variation in enzymes and enzyme systems due to acclimation to temperatures approximately 15° C apart is of a large magnitude. In this investigation differences of 40% have been found for brain ChE levels in *Fundulus heteroclitus.* Kanungo & Prosser (1959) find a 43% increase in oxygen consumption of liver homogenates of cold-adapted goldfish, *Carassius auratus,* and Precht (1951) finds a 50% decrease in succinodehydrogenase activity in the muscle of the fresh water eel, *Anguilla vulgaris,* adapted to warm temperatures.

Acclimation to thermal stress in poikilotherms can be measured by following the response of any of a number of physiological processes, and Precht (1959) has shown that movements of the gill covers of the swordtail, *Xiphophorus helleri,* and the guppy, *Lebistes reticulatus,* exhibit a significant adaptation to heat and cold. Freeman (1950) has shown that opercular rhythm, oxygen consumption and brain metabolism adapt together in response to thermal stress in the goldfish. In this investigation the changes in response to thermal stress were determined daily for opercular rate and brain ChE activity of the killifish. The normal opercular rate of approximately 100 beats per minute found in cold adapted fish was abruptly raised to over 230 beats per minute as a result of thermal shock caused by increasing the environmental temperature from 12 to 30° C. The opercular rate at the elevated temperature, over a period of five days, gradually returned to the cold-adapted level of 100 beats per minute, and coincided with the daily observed reduction in ChE activity (Text-fig. 3).

In response to heat stress, about 7 days are required to reach a stable lowered brain ChE activity level in *Fundulus heteroclitus.* Wells (1935) has shown that heat adaptation in the Pacific killifish, *Fundulus parvipinnis,* requires at least eight days for completion. In response to cold stress, a "lag" period of about four days occurs, followed by an increase in ChE activity with the development of a new stabilized enzyme level in about eleven days. Thus, from the standpoint of time, it is observed that it is more difficult to adapt biochemically to a decrease than to an increase in ambient temperature (Text-figs. 2, 3). Experimentally, it has also been found that it is more difficult to adapt a fish to a drop than to an increase in environmental temperature (Brett, 1956).

When the activity of fish brain ChE is plotted at the temperature of acclimation it becomes clear that a homeostatic mechanism exists in the central nervous system of *Fundulus heteroclitus* which regulates the level of ChE activity. The initial effect of heat stress on this enzyme system is to increase the specific activity of the enzyme ChE. By stressing a fish living at 12° C with a temperature of 30° C, the ChE activity will have a Q_{ChE} value of over 50, which is almost double the enzymatic activity present at the lower temperature. The effect of cold stress is opposite to that of heat stress, with the specific activity level of brain ChE falling below $Q_{ChE}=31$. As seen in Text-fig. 4 the response of the central nervous system of the killifish to thermal stress is to re-establish the previous enzyme activity level at the new ambient temperature. This process occurs within and coincides with the observed periods of acclimation to temperature variations. In the killifish the activity level of brain ChE that is maintained at each ambient temperature is approximately $Q_{ChE}=31$.

The seasonal increase in brain ChE levels in the killifish under conditions of constant environmental temperature has been described (Text-fig. 5). Considering the possible survival value of this enzyme in response to thermal shock, it is interesting that Hoar (1955) finds that the high and low lethal temperature limits of goldfish exhibit a seasonal cycle even under conditions of constant temperature. Wells (1935) found a seasonal variation in oxygen consumption of the Pacific killifish when kept at a constant temperature. Oxygen consumption rose in late January and early February to approximately 130% of the August through January mean and this rise in oxygen consumption occurred during that period of time when the lowest yearly water temperatures were recorded under natural conditions. The seasonal increase in brain ChE found in this investigation for *Fundulus heteroclitus* also occurred at the time of minimal recorded water temperatures in the fishes' natural environment. This fact, and the parallel increase in function (130%) occurring during the same four-week interval as that of the Pacific killifish, suggests that seasonal variations in physiology and biochemistry occur widely and may be important to poikilotherms that occupy environmental niches that are characterized by large seasonal variations in temperature.

Inactivation of brain and peripheral nerve ChE with specific anticholinesterases results in autointoxication due to the presence of unhydrolyzed acetylcholine at the synaptic junction. Under conditions of constant temperature, the residual quantities of brain ChE activity compatible with survival in fish, using anticholinesterase drugs, have been found to range between 20 and 60% of the normal level (Weiss, 1958, 1959, 1961). Nachmansohn & Feld (1947) have found an average value of 25% for a number of animals ranging between 10 and 50% of the normal activity level for this enzyme. The results of this investigation have indicated that the specific activity of brain ChE at any acclimation temperature is a constant as a result of biochemical adaptation. Thus, it is possible to calculate the ChE activity at the upper and lower lethal temperature if the Q_{10} and Q_{ChE} are known for any acclimation temperature. For the goldfish these values are 1.40 and 41 respectively (Table I). Fry *et al.* (1942) has found that goldfish acclimated to 20, 30 and 40° C have low lethal temperatures of 1, 9 and 17° C respectively. The calculated residual brain ChE activity at these temperatures are 52, 50 and 47% of the acclimated enzyme activity. The cold-blocking temperature of a simple tail reflex in goldfish acclimated to 25 and 35° C has been reported to be 5 and 10° C respectively (Roots & Prosser, 1962). In this case also, calculation of the residual enzyme activity indicates that this reflex activity stops when approximately 50% of the ChE activity has been lost.

The similarity in range of values for residual brain ChE activity due to both thermal and drug inactivation suggests that the primary cause of cold death and nerve block in this fish is due to failure of the central nervous system by physiological limitations set by experimentally induced or seasonal variation in ChE activity. The observed characteristics of cold death syndrome, such as numbness, muscular twitching, convulsion and paralysis (Fries, 1952; Roots & Prosser, 1962), are all indicative of physiological lack of this enzyme.

SUMMARY

1. In response to experimentally-induced thermal stress in the killifish, *Fundulus heteroclitus*, changes in brain cholinesterase (ChE) activity levels were observed. These alterations in enzymatic activity vary inversely with the temperature of acclimation.

2. The observed changes in brain ChE activity in the killifish occur in such a way that a specific activity level of $Q_{ChE}=31$ (milligrams of substrate hydrolyzed per hour by the enzyme present in 100 milligrams of tissue) is maintained regardless of the ambient temperature. This is achieved after a period of acclimation indicating that a homeostatic regulatory mechanism exists in brain tissue governing the activity of this enzyme.

3. Differences in *in vitro* stability of brain ChE in various fish species were found in response to elevated temperatures. Warm water species seem to have a more heat-stable enzyme than cold water forms.

4. Exposure to high temperature sufficient to cause death in the killifish does not result in denaturation of brain ChE, indicating that the lability of this enzyme *in vitro* does not occur *in vivo* and that the inactivation of this enzyme cannot be considered to contribute to heat death.

5. Cold death in fishes may be due to physiological limits set by the enzyme ChE of nerve tissue. Thermal inactivation of this enzyme may cause autointoxication due to the accumulation of unhydrolyzed acetylcholine at the synaptic junction.

6. The Q_{10} or factor for the increase in enzymatic activity for a 10° C rise in temperature *in vitro* for brain ChE from various fish species is apparently a species characteristic. Q_{10} values range from 1.02 to 1.45. The enzyme from warm water fish seems to be inactivated more easily by low temperature than brain ChE from cold water species.

7. A seasonal variation in brain ChE has been observed in the killifish under conditions of constant temperature. A 30% rise in enzyme activity occurs shortly after the lowest yearly water temperatures have been recorded in the natural habitat of this fish.

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3

Notes on a Collection of Bats from Central America, with the Third Record for *Cyttarops alecto* Thomas

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(Text-figures 1-10)

THIS PAPER presents records and life history data from three collections of bats from Central America: 48 specimens taken by the senior author, together with Priscilla Starrett and Thomas M. Uzzell, Jr., in the summer of 1957, during a three-month trip undertaken primarily for the purpose of collecting herpetological specimens; four bats collected by Arnold Menke and Fred S. Truxal, also in the summer of 1957, while engaged in collecting insects (Los Angeles County Museum Costa Rican Entomological Expedition); and 22 specimens collected in Costa Rica by members of the 1939 Allan Hancock Expedition aboard the "Velero III" (Fraser, 1943a, 1943b). All except eight of the bats were preserved as alcoholic specimens and all are now in the mammal collection of the Los Angeles County Museum. All of them have in common the fact that they were collected opportunistically, during the course of other types of investigation and collecting. The significance and interest of several of these specimens indicate the possible importance of even one or two bats which might be collected by any person carrying on non-chiropteran studies in the Neotropics, not to mention the potential which could be realized by a planned project of investigation of the bats of a neotropical region or country.

Abbreviations and locality names used in reference to specimens collected by the senior author and companions in 1957 are:

I.I.C.A.=Instituto Interamericano de Ciencias Agrícolas, under the Organization of American States, with headquarters and main Costa Rican facilities in Turrialba, Costa Rica.

I.T.I.C.=Instituto Tropical de Investigaciones Científicas, University of El Salvador, San Salvador.

S.T.I.C.A.=Servicio Técnico Interamericano de Cooperación Agrícola, a cooperative organization involving the United States and Costa Rican governments.

La Cinchona=S.T.I.C.A. quinine plantation near Varablanca, on the northeast slope of Volcán Poás.

Los Diamantes=S.T.I.C.A. experimental rubber station on the old rail line, near Guápiles, Costa Rica.

El Hogar Bíblico=Methodist children's home on the southwest slope of Volcán Barba, located at San José de la Montaña, several kilometers from the village of Barba, Costa Rica.

La Hulera=Formerly a S.T.I.C.A. experimental rubber station in Turrialba, Costa Rica, now a part of the I.I.C.A.

ACKNOWLEDGMENTS

During the course of the 1957 trip by the senior author and companions, a number of people were most gracious in their contributions to the success of our venture. Special appreciation is due here to some of these for their parts in making the collection of bats possible: Dr. Arístedes Palacios, Director, and Sra. Aida Cabezas O., Administrative Assistant, I.T.I.C., San Salvador; Sr. Alejandro Salazar, our host at Hacienda La Cumplida, Matagalpa, Nicaragua; Sr. Gerardo Budowski, Renewable Resources Department, I.I.C.A., Turrialba; Sr. Edilberto Camacho, La Hulera, Turrialba; Mr. and Mrs. Robert Bartlett, Los Diamantes; and Mr. and Mrs. William Brown, Hogar Bíblico, San José de la Montaña.

Parasite identifications were made by Richard S. Casebeer, University of Southern California

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(Arachnida), Walter E. Martin, University of Southern California (endoparasites) and Rupert L. Wenzel, Chicago Natural History Museum (Diptera). Fred S. Truxal, Los Angeles County Museum, identified certain insect remains from gut contents.

The illustrations were prepared by Miss Alice E. Boatright, Scientific Illustrator, Department of Zoology, University of Illinois. Miss LaVerne Curry, Alhambra Medical Center, Alhambra, California, prepared the serial sections of testes.

We wish also to thank Kenneth E. Stager and Charles A. McLaughlin for permission to include the Menke-Truxal specimens in this report.

All specimens are adult and fluid-preserved unless otherwise noted. All measurements are given in millimeters.

SYSTEMATIC LIST

Family EMBALLONURIDAE

Subfamily Emballonurinae

Saccopteryx bilineata (Temminck)

Urocryptus bilineatus Temminck, 1838-39, Tidjschr. natuurl. Gesch. Phys., 5:33—Surinam.

S. bilineata centralis Thomas, 1904, Ann. Mag. Nat. Hist., ser. 7, **13**:251—Teapa, Tabasco, Mexico.

Specimens.—Costa Rica: Prov. Puntarenas, Golfito; 1 female.

Reproduction.—This specimen showed no macroscopic evidence of reproductive activity. The wing sacs were not developed and lacked apparent glandular activity.

Food.—The esophagus and stomach were distended with finely chewed insect remains; several boluses of insect remains were also found in the lower small intestine.

Parasites.—Chiggers (larval Trombiculidae) were found embedded in the ears and at the base of the interfemoral membrane on the dorsal surface.

Remarks.—This specimen was captured in an insect net by Menke and Truxal as it flew back and forth in the breezeway of a building of the United Fruit Company facility, on the evening of July 17, 1957.

Measurement.—Forearm, 46.8.

Peropteryx kappleri Peters

Peropteryx kappleri Peters, 1867, Monatsb. Preuss. Akad. Wiss. Berlin, 1867: 473—Surinam.

Specimens.—Costa Rica: Prov. Puntarenas, "Gulf of Dulce" (Golfo Dulce), near Matapalo Head, Osa Peninsula (Fraser, 1943a: 160), at Station 940, Lat. 8° 24′ 30″ N., Long. 83° 17′ 05″ W. (Fraser, 1943b: 332, 407, Chart 1017); 10 males (3 skin and skull, 1 skeleton only), 12 females (4 skin and skull).

Reproduction.—Nine of the 12 females contained embryos; those in alcohol all contained male fetuses ranging from 19.0 to 22.3 mm. crown-rump length. The smallest fetuses lacked fur; the others showed fur dorsally and had hairs beginning to erupt in other regions. The testes of the fluid-preserved males were abdominal or inguinal, measured 1.9-2.5 × 2.6-3.1 mm. and showed activity ranging from early spermatogenesis with little sperm in the epididymides to active spermatogenesis with large amounts of sperm in the tubules and epididymides. The wing sacs of the males were well developed, with openings 5.5 mm. in length from the leading edge of the antebrachial membrane, and showed evidence of glandular activity. The sacs of the females were the same length as those of the males, but without any apparent glandular activity.

Food.—The intestines contained small amounts of finely chewed insect remains as well as small boluses containing bat hairs (presumably from the animals themselves).

Remarks.—These bats were collected above the high water line in a cave in "The large basaltic boulders near Matapalo Head" (Fraser, 1943a: 160), March 26, 1939.

Measurements.—Forearm, average for nine males, 45.6 (45.1-46.9), for 12 females, 49.5 (47.9-50.6); greatest length of skull, four males 15.3-16.1, four females, 15.9-16.1.

Subfamily Diclidurinae

Cyttarops alecto Thomas (Figures 1-10)

Cyttarops alecto Thomas, 1913, Ann. Mag. Nat. Hist., ser. 8, **11**:134—Mocajatube, near Pará, Brazil.

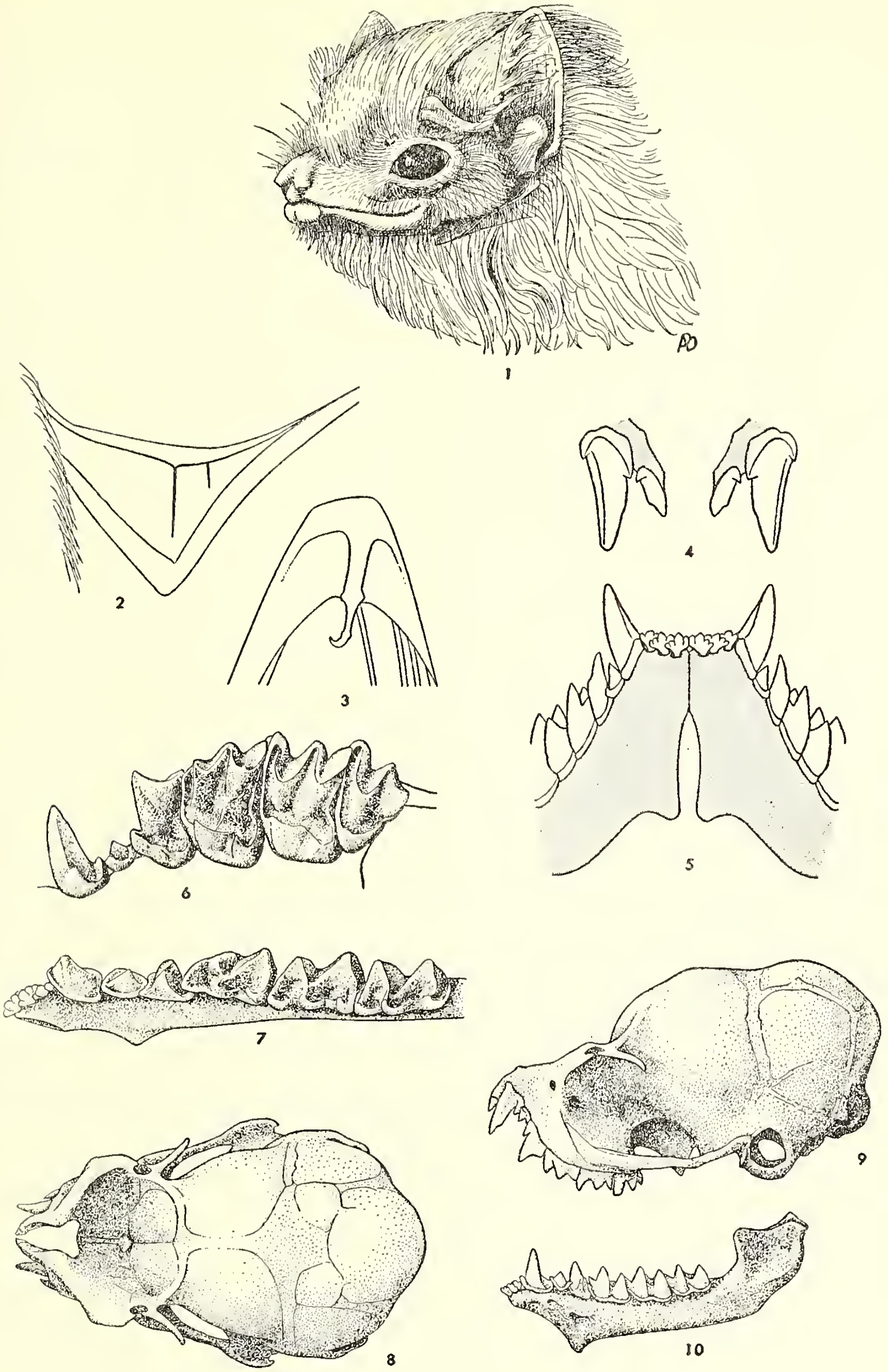
Specimens.—Costa Rica: Prov. Limón, Los Diamantes, 300 m. elevation; 1 male, 2 females.

Reproduction.—Neither female showed macroscopic signs of reproductive activity, either in condition of the uterus or in the appearance of the mammae. The male had small abdominal testes, 1.0 × 1.7 mm., which showed some spermatogenesis but no sperms in the tubules or epididymides.

Food.—The stomachs and intestines of these specimens contained a mixture of finely chewed insect remains.

Parasites.—No parasites were found in or on any of these specimens.

Remarks.—The specimens were given to the senior author by Mr. Robert Bartlett, director of the S.T.I.C.A. rubber station at Los Diamantes.



TEXT-FIGS. 1-10. *Cyttarops alecto* Thomas. **1.** Head, × 3. **2.** Antebrachial membrane, × 1¼. **3.** Detail of thumb, × 3. **4.** Upper incisors and canines, × approx. 20. **5.** Anterior view of mandibles, × approx. 20. **6.** Detail of upper tooth row, × approx. 12. **7.** Detail of lower tooth row, × approx. 9. **8.** Dorsal view of skull, × approx. 5. **9.** Lateral view of skull, × approx. 5. **10.** Lateral view of left mandible, × approx. 5.

They were brought to him on approximately August 1, 1957, by workers who had taken them from a group of bats (total number unknown) in an ornamental coco palm.

Nothing has been published concerning this bat since the original description of the genus and species in 1913. At that time (Thomas, 1913), two specimens were known: the type from Pará, Brazil, and a second specimen from Mazaruni River, British Guiana. The three specimens here recorded thus provide the third record of this bat and extend its known range northward into Central America.

Superficially, *Cyttarops* is similar in size and general appearance to *Peropteryx* or *Balantiopteryx*. However, closer examination of the skull and external features bears out Thomas' (1913) characterization of this bat as a relatively unspecialized diclidurine, intermediate in structure between *Diclidurus* and other New World emballonurids. Externally, *Cyttarops* differs from all American members of subfamily Emballonurinae in its low rounded ears (Text-fig. 1) and its broad antebrachial membrane which extends to the base of the distal phalanx of the thumb (Text-fig. 3). There is no antebrachial sac in either sex, nor is there any indication of one (Text-fig. 2). The interfemoral membrane is well developed and is supported from each ankle by a long (± 16 mm.), strong calcar. As in other emballonurids, the tail is attached to the under surface of the membrane for most of its length until, near the middle of the membrane, it turns dorsad and appears on the dorsal surface as a "free tail" of about four or five millimeters in length. There are no special modifications of the interfemoral membrane in either sex such as are found in *Diclidurus*. The fur of *Cyttarops* is long and silky with the color above and below appearing as Thomas described it, "uniform dull smoky grey," almost black. Externally, *Cyttarops* can be distinguished from the species of *Diclidurus* by its small size, dark color and unspecialized interfemoral membrane.

The skull of *Cyttarops* (Text-figs. 8, 9) closely resembles that of *Diclidurus*, except in the smaller size of the former. It differs also in having a more extremely pronounced frontal cup and a relatively long (2.5 mm.) postorbital process. The dental formula is $\frac{1\text{-}1\text{-}2\text{-}3 \times 2 = 32}{3\text{-}1\text{-}2\text{-}3}$ teeth, as in other New World Emballonuridae. The upper incisor (Text-fig. 4) comprises a major cusp and a small lateral basal cusp; the major cusp has a terminal (medial) lobe and a slightly less well developed subterminal (lateral) lobe. The upper canine (Text-fig. 4) has two basal cusps, one anterolingual and the other posterolabial in position. The shape and structure of the upper premolars and molars are shown in Text-fig. 6. The lower incisors are basically trilobed, with the lateral lobe of the first and second bipartite and of the posterior incisor simple. The lower canine has one anterior basal cusp and two posterior ones arising from the cingulum. The lower premolars and molars are shown in Text-fig. 7.

The features of the postcranial skeleton listed by Miller (1907:94-95) as characters of the subfamily Diclidurinae are seen in *Cyttarops:* the clavicle is expanded and the tibia is grooved longitudinally, although neither character is as well developed in *Cyttarops* as in *Diclidurus*.

Measurements.—Females (male damaged and not measured): forearm, 46.4, 47.2; metacarpal III, 44.3, 47.7; metacarpal IV, 36.2, 33.4; metacarpal V, 30.8, 33.5; (cranial measurements, largest female) greatest length of skull, 13.6; condylobasal length, 12.5; postorbital constriction, 3.8; zygomatic breadth, 8.0; mastoid breadth, 7.4; length of maxillary tooth row, 5.3; breadth across bases of upper canines, 2.4; maxillary breadth, including molars, 4.8; length of mandibular tooth row, 5.8; length of mandible, 9.9.

Family PHYLLOSTOMIDAE

Subfamily Phyllostominae

Phyllostomus hastatus panamensis J. A. Allen

Phyllostomus hastatus panamensis J. A. Allen, 1904, Bull. Amer. Mus. Nat. Hist., **20**:233.—Boquerón, Chiriquí, Panamá.

Specimens.—Costa Rica: Prov. Cartago, Turrialba, I.I.C.A. 602 m. elev.; 2 males.

Reproduction.—The testes of one animal were just entering the inguinal canal and measured 2.9 × 4.6 mm., those of the other were scrotal in position and measured 3.9 × 8.1 mm. Sections of testes from both animals showed early spermatogenesis with many interstitial cells still present, but no sperm in the tubules or epididymides.

Food.—The stomachs and intestines of these animals contained fruit pulp, insect remains, a few tiny bird feathers and sand grains. One also had in its intestine a partially digested tick, *Amblyomma ?cajennense* (Fabr.) (Ixodidae).

Parasites.—Four batflies, *Trichobius mixtus* Curran (Streblidae), were taken from the fur of these animals, which were heavily infested with this parasite as well as with mites (Acarina: Spinturnicidae). The tick mentioned above, which has previously been recorded only from terrestrial hosts, raised the question as to whether it was plucked from the animal's own body or was ingested with some small mammal eaten by the bat. Both bats had a small number of nematodes *(Histiostrongylus octacanthus* Lenta & Freitas) in the small intestine.

Remarks.—These animals were caught in a mist net set across a path in a platano plot behind the main building of the I.I.C.A., July 23 and 24, 1957. They were vicious and vociferous, producing a low, almost un-bat-like sound when being removed from the net.

Measurements.—Forearm, 90.6, 94.2; (cranial measurements for the larger male only) greatest length of skull, 40.8; condylobasal length, 37.0; palatilar length, 16.4; interorbital constriction, 7.6; zygomatic breadth, 22.0; mastoid breadth, 20.4; breadth of braincase, 15.1; length of maxillary tooth row, 14.5; maxillary breadth, including molars, 14.8; length of mandibular tooth row, 16.6; length of mandible, 27.8.

Subfamily Glossophaginae

Glossophaga soricina leachii (Gray)

> *Monophyllus leachii* Gray, 1843, *in* The Zoology of the Voyage of H.M.S. Sulfur . . . Vol. 1, No. 1, Mammalia, pt. 1, p. 18. —Realejo, Chinandega, Nicaragua.

Specimens.—El Salvador: Depto. San Salvador, San Salvador; 1 male, 2 females; Depto. Cuscatlan, 6.1 km. W. of Cojutepeque along the Interamerican Highway; 1 male, 5 females. Honduras: Depto. Valle, 5.0 km. from El Salvador border along Interamerican Highway, 1 female. Nicaragua: Depto. Matagalpa, Hda. La Cumplida; 1 male. Costa Rica: Prov. Cartago, Turrialba; 3 females; Prov. Heredia, San José de la Montaña, 1520 m. elev.; 1 female; Prov. Limón, Los Diamantes; 1 female.

Reproduction.—The seven females from El Salvador (July 7-9) each contained single embryos which ranged in crown-rump length from 4.5 to 12.4 mm. (the last nearly term, apparently); the two males from this country (July 9) had testes measuring 1.4 × 1.9 mm. and 2.6 × 3.6 mm., and showed early spermatogenesis and active spermatogenesis with sperm in the tubules and epididymides, respectively. The female from Honduras (August 16) carried an 11.6 mm. embryo, while the male from Nicaragua (July 16) had small testes, measuring 1.2 × 2.0 mm., which showed evidence of early spermatogenesis but no sperm in the tubules or epididymides. Of the three females from Costa Rica (July 2-August 9), only two exhibited evidence of reproductive activity: one had a swollen vulva with no other indications, the second had an enlarged uterus which, however, showed no macroscopic embryos. Further evidence of reproductive activity is shown in our collection by young animals taken July 8, in El Salvador, and July 27 and August 9, in Costa Rica. Felten (1956a) records pregnant females of this species in El Salvador from November through March and from July through September, and females with young from about the same months. Reproductively active males he reports from January, May and June and August through November. Bloedel (1955) reports a pregnant female *Glossophaga* from Panamá in February. Although these data might indicate a two-season breeding cycle, it seems more likely that these tropical bats continue to be reproductively active, at least as a species, throughout the year in Central America.

Food.—Fruit "pulp" and seeds of a number of different kinds of plants were present to some extent in the digestive tract of every *Glossophaga.* No pollen was found in any individual. Eight of the specimens, including representatives from all four countries in which this species was collected, had insect remains in their digestive tracts. In two cases the insect parts made up the bulk of the contents of the tract. The insects had been finely chewed but lepidopteran scales were readily recognizable, as well as portions of the wings of Diptera and Hymenoptera. There were also unidentifiable legs, antennae, setae, tracheae and mandibles. In this regard, it is of interest that Felten (1956a) found that captive *Glossophaga* in El Salvador preferred insects to honey-water or ripe banana. However, the specimen from Los Diamantes, Costa Rica, was captured at a bunch of ripe bananas with the ends cut off, which had been hung out for the purpose of attracting bats. During one evening several of these bats were observed to come, one bat at a time, and hang upside down, with wings folded, from the side of the fruit so as to reach the cut ends. An examination of the bananas in the morning showed cup-like excavations in the cut ends where the bats had rasped out the pulp with their tongues.

As might be expected, those bats netted early in the evening or taken from roosts late in the day had little food in their digestive tracts, whereas those netted later in the night had food in their stomachs and the animals taken in the morning had food material in the stomachs and intestines.

Parasites.—Only one *Glossophaga,* a specimen from Turrialba, Costa Rica, yielded a batfly: one specimen of *Trichobius uniformis* Curran (Streblidae) was found on this animal. In addition, an animal from Turrialba had several nematodes *(Capillaria* sp.) in the stomach and in the peritoneal cavity; a second animal, from San José de la Montaña, had a large number of unidentifiable nematodes in the small intestine.

Remarks.—This species was captured in the greatest variety of day roosts and habitats, as compared with the other species covered in this paper. *Glossophaga* were found in culverts (5-6 individuals) beneath the highway (El Salvador, Honduras), in the subcellar (colony of 30-50 individuals) of the main building of the I.I.C.A. (Costa Rica), in the eaves-attic (colony of several hundred individuals—apparently all the same species) of La Hulera (Costa Rica) and in a small, shallow cave (4 or 5 individuals; San José de la Montaña, Costa Rica). They were netted over a stream (Nicaragua), in a small banana plot (El Salvador) and at banana bait in a carport (Los Diamantes, Costa Rica). General habitats ranged from dry tropical forest at sea level in Honduras to Caribbean lowlands tropical wet forest and low montane wet forests (1520 m.) in Costa Rica.

Measurements.—Forearm, (two males) 32.8, 35.3; (14 females, average and range) 35.6 (34.1-38.0).

Subfamily Carolliinae

Carollia perspecillata azteca Saussure

> *Carollia azteca* Saussure, 1860, Rev. Mag. Zool., ser. 2, **12**:480.—Type locality subsequently fixed as Pérez, Veracruz, Mexico, by Dalquest (Occ. Pap. Mus. Zool., La. State U., No. 23, p. 2, 1950).

Specimens.—Nicaragua: Depto. Matagalpa, Hda. La Cumplida; 1 male. Costa Rica: Prov. Cartago, Turrialba; 1 female; Prov. Puntarenas, Golfito; 2 males.

Reproduction.—The female from Costa Rica carried an embryo, 13.9 mm. in crown-rump length. The male from Nicaragua had scrotal testes, measuring 3.8 × 6.8 mm., which showed active spermatogenesis with sperm in the tubules and epididymides. The males from Costa Rica had inguinal testes which measured 2.1 × 3.5 mm. and 4.6 × 7.5 mm. and showed early spermatogenesis with no sperm present in tubules or epididymides and active spermatogenesis with sperm in tubules and epididymides, respectively.

Of 28 females of this species taken in El Salvador in the months of October, November, December and March, Felten (1956a) found none pregnant. He records reproductively active males from November and April, and young animals in October and April. Enders (1935) mentions a pregnant female *Carollia perspicillata* from Panamá in March. It is possible that this species may breed all year round, as a species, as Bloedel (1955) suggested, but available evidence does not yet make this certain.

Food.—The digestive tracts of all four specimens contained several types of fruit pulp, seeds and vegetable fibers.

Parasites.—Single batflies, *Trichobius dugesii* Townsend (= *blandus* Curran) (Streblidae) were taken from the specimen from Nicaragua and one of the animals from Costa Rica. A mass of unidentifiable nematodes was found in the mesentery of one bat from Costa Rica, and a single unidentified worm was taken from the small intestine of another from the same country.

Remarks.—The two specimens from Golfito, Costa Rica, were captured by Fred Robinson in banana leaves, along with the specimen of *Thyroptera tricolor* mentioned below.

Measurements.—(Measurements of the specimen from Nicaragua precede those of specimens from Costa Rica; the Golfito males are listed last). Forearm, 43.7, 43.2, 43.1, 41.8; metacarpal III, 43.8, 42.6, 40.0, 40.1; metacarpal IV, 42.9, 41.2, 39.5, 38.7; metacarpal V, 44.3, 43.2, 40.1, 40.1; (cranial measurements for specimen from Nicaragua and female from Costa Rica) greatest length of skull, 24.0, 23.6; condylobasal length, 21.8, 21.2; palatilar length, 10.1, 10.0; interorbital breadth, 6.1, 6.2; postorbital constriction, 5.6, 5.2; mastoid breadth, 12.1, 11.2; length of maxillary tooth row, 8.2, 7.7; maxillary width, including molars, 8.4, 8.2; length of mandibular tooth row, 8.8, 8.5; length of mandible, 15.5, 15.6.

Carollia subrufa (Hahn)

> *Hemiderma subrufum* Hahn, 1905, Proc. Biol. Soc. Wash., **18**:247—Santa Efigenia, an hacienda 8 miles NW of Tepanatepec, near west coast of Oaxaca, Mexico.

Specimens.—El Salvador: Depto. San Salvador, San Salvador; 1 male; Depto. Cuscatlan, near Cojutepeque; 1 male.

Reproduction.—Both males had scrotal testes which showed late stages of spermatogenesis, with many interstitial cells and few sperms in the tubules and epididymides. The testes measured 2.0 × 3.6 and 3.2 × 5.2 mm., respectively. Felten (1956a) records pregnant females, in El Salvador, in February, March and October; no pregnancies in females from September, November and December. He took reproductively active males in January, March, August and December; found no activity in males from February and September through November. Young animals he found in April, September and October.

Food.—Several types and colors of fruit pulp were taken from the digestive tracts of both specimens, along with bat hairs. A small stalked inflorescence was also found in the small intestine of one, and a segment of an insect leg in the tract of the other.

Parasites.—One of the bats had several unidentifiable nematodes in the small intestine.

Remarks.—Although Felten (1956a) regards *Carollia subrufa* as a subspecies of *C. castanea* H. Allen, these two forms are readily distinguishable on the basis of external, cranial and dental characters, and should be considered as distinct species.

Measurements.—Forearm 38.6, 38.6; metacarpal III, 38.0, 37.4; metacarpal IV, 36.6, 36.3; metacarpal V, 38.1, 38.1; greatest length of skull, 21.7, 21.5; condylobasal length, 19.7, 19.5; palatilar length, 9.2, 9.1; interorbital breadth, 5.9, 5.8; postorbital constriction, 5.2, 5.2; mastoid breadth, 10.6. 10.7; length of maxillary tooth row, 7.1, 6.8; maxillary width, including molars, 7.7, 8.0; length of mandibular tooth row, 7.7, 7.6; length of mandible, 13.8, 14.0.

Subfamily Sturnirinae

Sturnira lilium parvidens Goldman

> *Sturnira lilium parvidens* Goldman, 1917, Proc. Biol. Soc. Wash., **30**:116.—Papayo, about 25 mi. NW of Acapulco, Guerrero, Mexico.

Specimens.—El Salvador: Depto. San Salvador, San Salvador; 1 male, 2 females. Nicaragua: Depto. Matagalpa, Hda. La Cumplida; 1 female.

Reproduction.—Signs of reproductive activity were present in all four specimens: the male (July 4) had greatly enlarged and scrotal testes (6.2 × 7.4 mm.), showing late spermatogenesis with sperms in the tubules and epididymides; two females, one from El Salvador (July 5) and the one from Nicaragua (July 13), had enlarged uteri and well developed mammae, indicating fairly recent parturition and lactation; the second female from El Salvador (July 8) had a moderately enlarged uterus and rather prominent mammae, possibly indicating early stages of pregnancy. No macroscopic embryos were found in any female. Felten (1956b) records of lactating female of this species in El Salvador in June.

Food.—All four specimens were presumably captured immediately after emerging from diurnal roosts, since none of them had any food in the digestive tract other than small amounts of fruit pulp in the lower colon. The Nicaragua specimens had a completely empty tract. Two specimens (male and female from El Salvador) were taken in a net at about 10:45 p.m., immediately after a heavy downpour which had started at dusk. The other two were extracted from nets in the morning.

Parasites.—No parasites were found on or in any of the specimens.

Remarks. — The specimen from Nicaragua marks the first record of this bat from that country.

Measurements. — (El Salvador male, two females and Nicaragua female, respectively) Forearm, 41.3, 38.8, 38.9, 38.4; metacarpal III, 39.5, 38.6, 38.7, 37.6; metacarpal IV, 40.1, 38.4, 38.3, 38.0; metacarpal V, 40.5, 39.1, 39.6, 39.1; greatest length of skull, 22.2, 20.7, 22.1, 21.1; condylobasal length, 19.7, 18.4, 19.5, 19.0; palatal length, 9.5, 9.1, 9.5, 9.0; interorbital breadth, 6.0, 5.3, 5.8, 5.9; postorbital constriction, 5.6, 5.1, 5.5, 5.6; zygomatic breadth, 13.1, 12.1, 13.1, 12.8; mastoid breadth, 11.7, 10.8, 11.9, 10.8; length of maxillary tooth row, 6.6, 6.3, 6.2, 6.3; maxillary width, including molars, 8.1, 7.4, 7.7, 7.5; length of mandibular tooth row, 7.4, 7.0, 7.2, 7.2; length of mandible, 14.3, 13.5, 13.9, 13.6.

Sturnira ludovici Anthony

> *Sturnira ludovici* Anthony, 1924, Amer. Mus. Nov., no. **139**:8. — Near Gualea, northwestern Ecuador, elevation about 4000 ft.
>
> *Sturnira hondurensis* Goodwin, 1940, Amer. Mus. Nov., no. **1075**:1-2. — La Cruz Grande, Dept. of La Paz, Honduras, elevation about 3000 ft.

Specimens—Costa Rica: Prov. Cartago, Volcán Turrialba, elevation 2820 m.; 2 males (1 imm.), 1 female.

Reproduction.—The immature male had abdominal testes which measured 1.9 × 3.0 mm., but showed signs of beginning spermatogenesis, with many interstitial cells and no sperms present. The adult male had scrotal testes, measuring 3.7 × 4.2 mm., and enlarged epididymides; the testes showed active spermatogenesis, with sperms in the tubules and some in the epididymides, and numerous interstitial cells. The female showed no macroscopic signs of reproductive activity. (All three specimens were captured July 24).

Food.—As with the *S. lilium* discussed above, these animals must have been captured shortly after emergence from their daytime roosts and prior to much feeding. Two of them had only small amounts of fruit pulp in the colon and the third had also a small amount of the same material in its stomach. Additional evidence of their having been captured early in the evening was shown by the extremely torpid condition of these animals when they were extracted from the net in the morning. The cold night had reduced their activity to the point where blowflies had laid eggs in the fur of one, presumably early in the morning. All three bats again became quite active during the trip back to the laboratory.

Parasites. — All three specimens of *Sturnira ludovici* were heavily infested with batflies, five of which were collected. These proved to be *Pterellipsis proxima* Seguy (Streblidae).

Remarks.—This species has been recorded previously from Costa Rica by two specimens from Agua Buena, Prov. Puntarenas, near the Panamá border.

Measurements.—(1 male, 1 female, respectively) forearm, 43.9, 42.9; metacarpal III, 42.4, 41.4; metacarpal IV, 42.5, 41.4; metacarpal V, 44.2, 43.0; greatest length of skull, 22.4, 22.4; condylobasal length, 20.4, 20.0; palatal length, 9.4, 9.0; interorbital breadth, 6.4, 6.0; postorbital constriction, 6.1, 5.7; zygomatic breadth, 13.0, 12.7; mastoid breadth, 11.0, 11.3; length of maxillary tooth row, 6.4, 6.4; maxillary width, including molars, 7.6, 7.8; length of mandibular tooth row, 7.4, 7.1; length of mandible, 14.5, 14.1.

Subfamily Stenoderminae

Vampyrops helleri Peters

Vampyrops helleri Peters, 1866, Monatsb. Preuss. Akad. Wiss. Berlin, **1866**:392.—"Mexico."

Specimens.—Costa Rica: Prov. Cartago, Turrialba; 1 female.

Reproduction.—This animal showed no macroscopic indication of reproductive activity (August 11).

Food.—Small amounts of fruit pulp were present in all parts of the digestive tract.

Parasites.—No parasites, external or internal, were found on this animal.

Remarks.—This species has been recorded previously in Costa Rica from two localities: Prov. Cartago, Guayalo (not far from Turrialba), by two specimens (Sanborn, 1955); Prov. Limón, Jiménez, by 1 specimen (Allen, 1893, and Goodwin, 1946). It is otherwise known in North America by but five additional specimens, taken in México, Honduras and Panamá (Sanborn, 1955).

For use of the generic name *Vampyrops* Peters, 1865, in preference to *Platyrrhinus* Saussure, 1860, see De la Torre and Starrett (1959).

Measurements.—Forearm, 38.8; metacarpal III, 38.5; metacarpal IV, 38.5, metacarpal V, 39.0; greatest length of skull, 22.8; condylobasal length, 20.4; palatal length, 9.8; interorbital breadth, 6.2; postorbital constriction, 5.7; zygomatic breadth, 12.1; mastoid breadth, 10.8; length of maxillary tooth row, 7.9; maxillary width, including molars, 8.9; length of mandibular tooth row, 8.7; length of mandible, 15.5.

Vampyressa thyone Thomas

Vampyressa thyone Thomas, 1909, Ann. Mag. Nat. Hist., (8) **4**:231.—Chimbo, near Guayaquil, Ecuador.

Vampyressa minuta Miller, 1912, Proc. U.S. Nat. Mus., **42**:25.—Cabima, Panamá.

Specimens.—Nicaragua: Depto. Matagalpa, Hda. La Cumplida, 670 m. elevation; 1 female.

Reproduction.—The uterus of this animal (July 14) contained an embryo of 11.8 mm., crown-rump length (in shrivelled condition; probably closer to 13.5 mm. in actual length).

Food.—A tiny amount of pulp was found in this bat's small intestine.

Parasites.—No parasites were found in or on this specimen.

Remarks.—We concur with Hershkovitz (1949) in placing *V. minuta* Miller in synonymy with *V. thyone* Thomas. The Nicaragua specimen compares favorably in measurements with those listed by Goodwin (1946) for the type of *V. minuta,* from Cabima, Panamá, and for a specimen from Agua Buena, Costa Rica. We also find the specimen from Nicaragua indistinguishable in salient characters, including measurements, from three specimens from Peru in the Chicago Natural History Museum collection.

This specimen marks the first record of *Vampyressa thyone* from Nicaragua, and extends the northern limit of range for the species to this country. The previous northermost record was provided by two specimens from Agua Buena, Prov. Puntarenas, Costa Rica (Goodwin, 1946).

Measurements.—Forearm, 31.4; metacarpal III, 30.3; metacarpal IV, 29.3; metacarpal V, 30.9; greatest length of skull, 18.4; condylobasal length, 16.6; palatal length, 8.6; interorbital breadth, 4.9; zygomatic breadth, 10.5; mastoid breadth, 9.0; length of maxillary tooth row, 6.0; length of mandibular tooth row, 6.4; length of mandible, 11.5.

Artibeus jamaicensis jamaicensis Leach

Artibeus jamaicensis Leach, 1821, Trans. Linn. Soc. London, **13**:75.—Jamaica.

Specimens.—Nicaragua: Depto. Matagalpa, Hda. La Cumplida; 1 male. Costa Rica: Prov. Cartago, Turrialba; 1 male.

Reproduction.—The animal from Nicaragua (July 17) had scrotal testes which measured 3.5 × 5.0 mm. and showed late spermatogenesis with numerous sperms in the tubules and some in the epididymides. The male from Costa Rica (July 24) had scrotal testes, 3.8 × 5.3 mm., which showed late spermatogenesis, with numerous sperms in the tubules and epididymides and few interstitial cells. Felten (1956b) records breeding males in October and lactating females in March, in El Salvador.

Food.—The intestines of these animals contained fruit pulp, plant fibers and bat hairs (presumably from the animals themselves). The animal from Costa Rica also had an ant (Formicidae: Ponerinae) embedded in a reddish amber-like substance in its intestine.

Parasites.—The only parasite found was an unidentifiable roundworm which was taken from the small intestine.

Measurements.—Forearm (specimens from Nicaragua and Costa Rica, respectively), 60.7, 64.7; (specimen from Nicaragua) metacarpal III, 57.0; digit III, first phalanx, 17.6; digit III, second phalanx, 30.7; metacarpal IV, 56.0; digit IV, first phalanx, 15.7; metacarpal V, 58.8; digit V, first phalanx, 11.5; postorbital constriction, 7.9; zygomatic breadth, 18.7; percentage postorbital constriction/zygomatic breadth, 42.3 (specimen from Costa Rica), 38.9.

Artibeus lituratus palmarum J. A. Allen & Chapman.

Artibeus palmarum J. A. Allen & Chapman, 1897, Bull. Amer. Mus. Nat. Hist., **9**:16.—Port of Spain, Trinidad.

Specimens.—El Salvador: Depto. San Salvador, San Salvador; 1 male (young adult), 1 female. Nicaragua: Depto. Matagalpa, Hda. La Cumplida; 1 male (imm.). Costa Rica: Prov. Cartago, Turrialba; 2 females (1 imm.).

Reproduction.—All three females showed signs of reproductive activity: that from El Salvador (July 8) had greatly developed mammae and an enlarged uterus, indicating recent parturition and lactation still in progress; one from Costa Rica (July 23) contained a 39 mm. (crown-rump length) male embryo which, judging from the development of the maternal mammae, was near term; the second from Costa Rica (July 22) had a swollen vulva and slightly enlarged uterus. The young adult male from El Salvador (July 6) had scrotal testes, 3.9 × 5.0 mm., showing active spermatogenesis, with some sperm in the tubules and a few in the epididymides, and few interstitial cells; the immature male from Nicaragua also had scrotal testes, 3.5 × 4.8 mm., but which showed only some spermatogenic activity with no sperms present, and many interstitial cells visible. Bloedel (1955) mentions a female that gave birth in March in Panamá: Hall & Jackson (1953) record a pregnant female in May, also in Panamá; Felten (1956b) mentions reproductive activity in males in October and November, a female with young in April, in El Salvador. Year round reproductive activity may be indicated for *Artibeus lituratus*, as a species, in Middle America.

Food.—Fruit pulp of several colors and types, plant fibers and bat hairs occurred in varying amounts in the digestive tracts of these bats. In addition, a few scattered insect remains were found in the intestines of two of the animals.

Parasites.—The only parasites found were batflies, *Paratrichobius longicrus* Ribeiro (Streblidae) and several tapeworms, *Vampirolepis* sp. (Hymenolepididae), all of which were taken from the male from El Salvador.

Measurements.—(Young adult male from El Salvador, adult females from El Salvador, Costa Rica, respectively) Forearm, 66.0, 70.2, 72.3; (young adult male, female from Costa Rica) metacarpal III, 62.6, 70.4; digit III, first phalanx, 21.2, 23.9; digit III, second phalanx, 34.3, 37.5; metacarpal IV, 62.6, 66.6; digit IV, first phalanx, 17.5, 19.9; metacarpal V, 64.9, 72.8; digit V, phalanx, 14.0, 15.8; greatest length of skull, 27.6, 31.5; condylobasal length, 24.5, 28.0; palatal length, 12.7, 15.1; interorbital breadth 7.4, 7.8; postorbital constriction, 6.0, 6.6; zygomatic breadth, 16.0, 19.6; mastoid breadth, 14.2, 17.0; length of maxillary tooth row, 9.8, 11.0; maxillary width, including molars, 11.5, 13.9; length of mandibular tooth row, 10.9, 12.3; length of mandible, 18.8, 21.6; percentage postorbital constriction/zygomatic breadth, 37.5, 33.7.

Family THYROPTERIDAE

Thyroptera tricolor albigula G. M. Allen

Thyroptera tricolor albigula G.M. Allen, 1923, Proc. New Eng. Zool. Club., **9**:1.—Gutiérrez, Chiriquí, Panamá.

Specimen.—Costa Rica: Prov. Puntarenas, Golfito; 1 male.

Reproduction.—The testes were small and were not sectioned (August 8).

Food.—Small amounts of insect remains were found in the stomach and colon of this specimen.

Parasites.—No parasites were found on or in this animal.

Remarks.—This specimen marks the second record for the species in Costa Rica and the fourth north of Panamá.

Measurements.—Forearm, 34.3.

Family VESPERTILIONIDAE

Subfamily Vespertilioninae

Myotis nigricans nigricans (Schinz)

> *Vesp (ertilio) nigricans* Schinz, 1821, Das Thierreich ......, **1**:179.—Fazenda de Aga, near Rio Iritiba, Espírito Santo, southeastern Brazil.

Specimens.—El Salvador: Depto. La Libertad, 2.7 mi. E of Colón; 2 males. Costa Rica: Prov. Alajuela, Volcán Poás, elevation approximately 1980 m.; 1 male.

Reproduction.—Reproductive activity was not indicated for any of these specimens. The female (July 4) showed no macroscopic signs of activity; the males both had external testes which were not descended into the scrotal areas, and the testes of both (El Salvador, July 4; Costa Rica, August 6) were small (2.0 × 3.3, 3.0 × 4.0 mm.) and showed little spermatogenic activity with no sperm present and numerous interstitial cells visible.

Food.—The digestive tracts of all three specimens contained the finely chewed remains of insects.

Parasites.—Two males of a batfly, *Basilia* sp. (Nycteribidae) were taken from one of the bats from El Salvador.

Measurements.—Forearm (male from Costa Rica last), 34.7, 35.6, 36.7.

Eptesicus brasiliensis propinquus (Peters)

> *Vesperus propinquus* Peters, 1872, Monatsb. Preuss. Akad. Wiss. Berlin, **1872**: 262.—Santa Isabel, Peten, Guatemala.

Specimens.—Costa Rica: Prov. Cartago, Turrialba; 1 male, 1 female.

Reproduction.—The female (July 28) showed no macroscopic signs of reproductive activity; the male (July 23) had small (2.7 × 4.7 mm.) scrotal testes which showed little spermatogenic activity with no sperm and numerous interstitial cells.

Food.—The digestive tracts of both specimens were filled with finely chewed remains of insects.

Parasites.—No parasites were found on or in either of the specimens.

Measurements.—(Male first) forearm, 40.1, 42.9; metacarpal III, 38.8, 40.5; metacarpal IV, 38.3, 39.9; metacarpal V, 37.2, 38.9; greatest length of skull, 16.5, ——; condylobasal length, 15.6, ——; palatilar length, 5.9, 6.1; postorbital constriction, 3.8, ——; zygomatic breadth, 10.8, ——; mastoid breadth, 8.5, ——; length of maxillary tooth row, 5.9, 6.1; maxillary width, including molars, 6.4, 6.9; length of mandibular tooth row, 6.6, 6.5; length of mandible, 11.7, 12.2.

Family Molossidae

Tadarida brasiliensis brasiliensis (I. Geoffroy-Saint Hilaire)

> *Nyctinomus brasiliensis* I. Geoffroy-Saint Hilaire, Ann. Sci. Nat., **1**:343.—Curityba, Paraná, Brazil.

Specimens.—Costa Rica: Prov. Heredia, San José de la Montaña, 1520 m. elev.; 1 male, 11 females; Prov. Alajuela, La Cinchona, approx. 1350 m. elev.; 3 males, 1 female.

Reproduction. — The only possible macroscopic indication of reproductive activity in the females (July 27, August 9) was a slight enlargement of the right horn of the uterus in five individuals; the males (July 27, August 9) had small (1.3-1.6 × 2.3-3.3 mm.) inguinal testes which showed no spermatogenesis, no sperms in the tubules or epididymides, and many interstitial cells.

Food.—Small amounts of insect remains were found in the lower digestive tracts of these bats, all of which were taken from roosts during the day.

Parasites.—Mites (indet.) were taken from one of the specimens from La Cinchona.

Remarks.—The specimens from San José de la Montaña were taken from a colony of unknown size under the roof of one of the buildings at the Hogar Bíblico; those from La Cinchona were collected from a colony of several hundred individuals under the loose clapboards on the side of a building.

Measurements.—Forearm, 4 males, av. 43.2 (41.6-44.0); 12 females, av. 44.0 (41.6-45.9).

Molossus major (Kerr)

> *V (espertilio) mol (ossus) major* Kerr, the Animal Kingdom, p. 97.—Martinique, Lesser Antilles.

Specimens.—El Salvador: Depto. San Salvador, San Salvador, I.T.I.C.; 2 males, 2 females.

Reproduction. — The only possible macroscopic indication of activity in the females (July 5) was a slight enlargement of the right horn of the uterus in both specimens; the testes of the male taken July 4 were small (2.3 × 4.0 mm.), scrotal, and showed active late spermatogenesis with sperms in the tubules and epididymides and relatively large number of medium-sized interstitial cells.

Food.—The stomach of one female was greatly distended with insect remains, the tracts of the other bats were less full of the same material.

Parasites.—No parasites were taken from any of these animals.

Remarks.—These four specimens were shot at dusk over the grounds of the I.T.I.C., San Salvador. Each evening, during our week's stay at the I.T.I.C., from between 6:15 to 6:30 and dark, large numbers of the bats flew low over the institute as they passed in a fairly direct path from southwest (the direction of San Salvador) to northeast. The full condition of the digestive tracts of the animals collected and the low and somewhat slow and erratic flight of the bats as they passed over the institute indicate that they were feeding as they left the roost, and the direct general orientation of flight suggests that they were possibly on their way to night feeding grounds and roosts in the cultivated regions around the city.

The present state of knowledge concerning the genus *Molossus* leaves the matter of relationships between named forms far from clear. Therefore, we are using here the oldest name which was applied to the pygmy form which our specimens represent.

Measurements.—Forearm (1 male, 2 females) 39.1, 38.5, 38.0.

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4

Control of the Snake Mite, *Ophionyssus natricis* (Gervais), in Captive Reptile Collections[1]

J. H. Camin[2], G. K. Clarke[3], L. H. Goodson[4] & H. R. Shuyler[5]

(Text-figures 1-3)

Introduction

Although the snake mite, *Ophionyssus natricis* (Gervais, 1844), has been known from captive reptiles since 1823 (Metaxa, 1823), it has only recently been found on wild reptiles (Yunker, 1956). Members of the genus *Coluber* and its close relatives appear to be its favorite hosts in zoological gardens throughout the world and in its natural habitat in Egypt, but it will readily attack most species of snakes and many species of lizards and will occasionally parasitize tortoises.

The snake mite is an obligate, blood-sucking ectoparasite. It is a typical nidicole, spending most of its time in the dark, moist cracks and crevices of the cage and coming to the host only to feed (Camin, 1953). At 25°C the eggs incubate in less than 48 hours and the non-feeding stages, the larva and deutonymph, each have a duration of less than 24 hours. The mites feed only as protonymphs and adults and these stages will endure for one to five weeks with or without a blood meal. However, the protonymphs are unable to molt into the deutonymphal stage and the adult females are incapable of producing eggs unless they have engorged on reptilian blood. Unmated females produce only male (haploid) eggs parthenogenetically, whereas mated females produce both male (haploid) and female (diploid) eggs in approximately equal numbers (Camin, 1953; Oliver, Camin & Jackson, 1963). The viability of all stages is adversely affected by humidities below the optimum of 95% R.H., but the effect is greater on the younger stages.

Directly or indirectly, *O. natricis* is an important cause, perhaps the primary cause, of snake mortality in zoos and other captive reptile collections around the world. Even light infestations will reduce the vigor and mar the appearance of captive reptiles. Infested snakes do not molt properly and often refuse to feed. Heavily parasitized snakes frequently die from exsanguination in a few weeks' time. In addition to such direct damage, these mites transmit some important reptile diseases, such as the highly virulent and often fatal hemorrhagic septicemia of snakes (Camin, 1948), caused by the bacterium *Aeromonas hydrophila* (Sanarelli, 1891). Although it is probably not the vector in nature, *O. natricis* is the primary vector of a haemogregarine disease of the lung and blood in captive snakes (Hull & Camin, 1960; Hull & Camin, in preparation) and there is suggestive evidence that this mite may also be capable of transmitting the filarial worm, *Macdonaldius seetae* Khanna, 1933, from snake to snake (Hull & Camin, 1959).

The primary goal of this investigation was to find an effective means of eradicating or control-

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[2] Professor of Entomology, University of Kansas, Lawrence, and consultant to MRI. This paper is listed as contribution no. 1227 from the Department of Entomology, University of Kansas.

[3] Director, Topeka Zoological Park, Topeka, Kansas; Laboratory Technician at MRI during the course of this investigation.

[4] Head, Biochemistry Section, Midwest Research Institute.

[5] Entomologist, Conservation Industries, Inc., Kansas City, Kansas, and consultant to MRI.

ling the snake mite on captive reptiles. Previous methods had all proved inadequate, being either ineffective or impractical. Some killed too few mites. Others killed too many snakes. Our aim was to find a method suitable for routine application that would eradicate the mites without harming their hosts and, if possible, without necessitating the removal of the reptiles from exhibit. Methods requiring the individual dipping or dusting of reptiles are generally impractical, especially with large collections. Dusts and compounds using oils as vehicles tend to mar the appearance of the cages and the reptiles and therefore cause extra work in maintenance. Consequently, water soluble compounds and aqueous suspensions of acaricides would be the materials of choice, if they would also meet the other requirements.

During the course of our investigation we learned of the excellent results obtained in the control of the snake mite with Dri-Die 67 by Dr. I. Barry Tarshis in California. This work was subsequently published (Tarshis, 1960, 1961a, 1961b). Our results with this material were also promising, although not as promising as those of Dr. Tarshis, and other substances appeared to be somewhat superior. This will be further discussed.

It must be stated at the outset that time and funds did not permit us to carry this investigation to completion. However, because of the dramatic results obtained with two of the acaricides that were tested, even though our remaining data are not as clear-cut or as conclusive as might be desired, we feel that all of our results should be made available to those who may have use for them.

Materials and Methods

In order to develop a method of snake mite control that would be applicable under the widely varying conditions that prevail in different reptile collections, it was decided that tests should be conducted under optimal conditions for the mites and suboptimal or adverse conditions for the snakes. An acaricide that is effective under such conditions should then be effective in any situation that might be encountered in practice. For this reason initial tests for acaricidal activity were conducted against engorged adult female snake mites at 23-26°C and 80-95% R.H. Previous experience has shown that the engorged adult female is much more tolerant to variations in environmental conditions than any of the other active stages. Because of limitations of time and the fact that the incubation period of snake mite eggs is so brief, it was not practical or fruitful to test for ovicidal action. In addition, the egg is followed by the short-lived larval stage, which is the most vulnerable of all the active stages. Therefore, if an acaricide is active for at least 48 hours, it will kill the emerging larvae, even if it does not attack the egg.

Preliminary screening of 45 potentially acaricidal materials (Table I) was accomplished by treating the inner surfaces of 45 petri dishes, each with a different substance, and then placing ten engorged female mites in each dish. Active, immobilized and dead mites were counted after the first half hour, at approximately hourly intervals for the next 8 to 18 hours, at approximately 24 hours and at approximately 24-hour intervals thereafter until 96 to 168 hours had passed or until all the mites were dead. It was observed that some of the mites in the untreated controls were dead after four to five days and therefore mortality in the experimental dishes could not be reliably attributed to acaricidal action of the test compound after 96 hours. Among the 45 materials were several inert dusts and solvents that serve as vehicles for dust and spray toxicants. These served as treated controls against which the toxic materials could be compared. Some of these had mild acaricidal effects and were not used as vehicles for the test compounds.

After this preliminary screening, substances that had killed all of the mites in 24 hours or less were further tested for toxicity to reptiles. Materials that had caused 100% mite mortality in 48, 72 and 96 hours were noted and set aside to be tested further, if the more rapidly killing acaricides should all prove to be toxic to reptiles. Because small snakes were unobtainable in quantity at the time that we were ready to begin testing for herpeticidal action, American "chameleons" or anoles (*Anolis carolinensis*) were used. These are small, relatively fragile lizards, which are generally available from Florida at any time of the year. Later the same formulations were also tested against ring-necked snakes (*Diadophis punctatus arnyi*), which varied from 8 to 14 inches in length, and against young garter snakes (*Thamnophis radix* and *Thamnophis sirtalis parietalis*), which ranged in length from 18 to 26 inches. Such animals were utilized because previous experience had demonstrated that materials that are harmless to small, delicate snakes and lizards are generally also safe for use on larger reptiles. On the other hand, substances that have appeared non-toxic to large reptiles have frequently proved to be harmful and sometimes even lethal to smaller individuals.

Five anoles were used in each of the lizard tests. These were each given water prior to the

TABLE I. COMPARATIVE ACARICIDAL ACTIVITY AND TOXICITY TO REPTILES OF VARIOUS PRODUCTS SCREENED

Product (Alphabetical by Classification)	Manufacturer or Formulator	Use Form	Dilution	Toxicity to Mites[1]						Toxicity to Reptiles[2]				
				Dosage	No. Dead/No. Tested 4hrs	24hrs	48hrs	72hrs	96hrs[3]	Dosage	Spec.[4]	No. Dead/No. Tested 24hrs	96hrs	168hrs[3]
ORGANIC CHEMICAL PRODUCTS														
Botanicals:														
Pyrethrins, Synergized[5]														
Pyrenone Roach Spray Concentrate 1.5% Pyrethrins 7.5% Piperonyl Butoxide	Fairfield (FMC)	Oil Solution	1:48 by vol.	5 drops	0/10	3/10	6/10 (41)	—	—	—	—	—	—	—
Pyrenone Dust Base, BP-13-30 0.6% Pyrethrins 6.0% Piperonyl Butoxide	Fairfield (FMC)	Dust	None	3 mg.	7/10	10/10 (20)	x	x	x	2 g.	RS	0/5	5/5 (72)	x
		Dust w/Attaclay X-250	1:10 by wt.	3 mg.	0/10	2/10	3/10 (28)	—	—	—	—	—	—	—
Rotenone[6]	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Carbamates:														
Hercules 5727, Tech. N-methyl m-isopropylphenyl carbamate	Hercules	Dust	None	3 mg.	10/10 (1)	x	x	x	x	—	—	—	—	—
Sevin, Tech.	Union Carbide	Dust w/Sulphenone 50W[7]	5%	3 mg.	3/10	6/10	—	—	—	—	—	—	—	—
Chlorinated Aryl Hydrocarbons:														
Lindane[8]	Hooker	—	—	—	—	—	—	—	—	—	—	—	—	—
DDT Relatives:														
Chlorobenzilate 25W 25% Wettable Powder	Geigy	Dust w/Attaclay X-250	1:800 by wt	3 mg.	0/10	3/10	3/10 (28)	—	—	—	—	—	—	—
Dimite 25% Emulsifiable Conc.	Acme Paint	H_2O Emulsion	1 ml/gal	5 drops	0/10	3/10	7/10	7/10	9/10	—	—	—	—	—
Kelthane W 18.5% Wettable Powder	Rohm & Haas	H_2O Suspension	9 g/gal	5 drops	0/10	0/10	0/10	1/10	2/10 (82)	—	—	—	—	—
			4 g/gal	5 drops	0/10	0/10	0/10	0/10	0/10 (82)	—	—	—	—	—
Heterocyclic Compound (not listed elsewhere):														
Bayer 30686 2,3-quinoxalinedithiol cyclic trithiocarbonate	Chemagro	Dust	3%	20 mg.	0/10	0/10	0/10	1/10	2/10	—	—	—	—	—
Nitrophenyl Compound:														
Bayer 28589 2,6-di-*tert*-butyl-4-nitrophenol	Chemagro	Dust	3%	20 mg.	0/10	0/10	0/10	0/10	1/10	—	—	—	—	—

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Product (Alphabetical by Classification)	Manufacturer or Formulator	Use Form	Dilution	Toxicity to Mites[1]						Toxicity to Reptiles[2]				
				Dosage	No. Dead/No. Tested					Dosage	Spec.[4]	No. Dead/No. Tested		
					4hrs	*24hrs*	*48hrs*	*72hrs*	*96hrs*[3]			*24hrs*	*96hrs*	*168hrs*[3]
ORGANIC CHEMICAL PRODUCTS														
Phosphorus Containing Compounds—Phosphorous Aliphatic Derivatives:														
Experimental Insecticide 18706 25% Oil Solution, 2#/gal.	Amer. Cyanamid	H_2O Emulsion	4 ml./gal.	5 drops	8/10	10/10 (6)	x	x	x	—	—	—	—	—
0,0-dimethyl-S-(N-ethyl carbamoyl methyl) phosphorodithioate			2 ml./gal.	5 drops	8/50	41/50	50/50 (45)	x	x	5 ml.	RS	0/10	0/10	0/10
DDVP (Vapona)	Shell	H_2O Emulsion	0.2%	5 drops	10/10 (1)	x	x	x	x	2 ml.	L	4/5	5/5 (66)	x
Dibrom 8E 8#/gal.; Emulsifiable Conc.	California Chem.	H_2O Emulsion	4 ml./gal.	5 drops	3/10	10/10 (22)	x	x	x	2 ml.	L	0/5	0/5	0/5
			4 ml./gal.	—	—	—	—	—	—	5 ml.	RS	0/5	1/5	1/5
			2 ml./gal.	5 drops	4/50	23/50	36/50	39/50	45/50	5 ml.	RS	0/10	0/10	0/10
Dylox 50% Soluble Powder	Chemagro	H_2O Solution	2 g./gal.	5 drops	5/10	8/10	10/10 (26)	x	x	—	—	—	—	—
Malathion E-5 5#/gal.; Emulsifiable Conc.	Thompson-Hayward	H_2O Emulsion	6 ml./gal.	5 drops	2/10	10/10 (22)	x	x	x	2 ml.	L	0/5	0/5	0/5
			6 ml./gal.	—	—	—	—	—	—	5 ml.	RS	5/5 (2)	x	x
			2 ml./gal.	—	—	—	—	—	—	5 ml.	GS	2/10	2/10	2/10
Systox Spray Concentrate 4#/gal.; Emulsifiable Conc.	Chemagro	H_2O Emulsion	5 ml./gal.	5 drops	2/10	5/10	6/10 (33)	—	—	—	—	—	—	—
Phosphorous Hetrocyclic Derivatives:														
Co-Ral 25W 25% Wettable Powder	Chemagro	H_2O Suspension	2 g./gal.	5 drops	0/10	4/10	4/10	4/10	7/10	—	—	—	—	—
Co-Ral Livestock Duster	Chemagro	Dust	0.5%	3 mg.	0/10	4/10	7/10 (33)	—	—	—	—	—	—	—
Delnav 47% Emulsifiable Conc.	Hercules	H_2O Emulsion	1:300 by vol.	5 drops	0/10	6/10	10/10 (28)	x	x	—	—	—	—	—
			1:600 by vol.	5 drops	11/50	17/50	19/50	19/50	21/50	—	—	—	—	—
Diazinon 20S 20% Oil Soluble Concentrate	Geigy	Oil Solution	1:192 by vol.	5 drops	0/10	5/10	7/10 (41)	—	—	—	—	—	—	—
Diazinon 25E 25% Emulsifiable Conc.	Geigy	H_2O Emulsion	1:240 by vol.	5 drops	3/10	10/10	x	x	x	2 ml.	L	0/5	0/5	0/5
			1:240 by vol.	—	—	—	—	—	—	5 ml.	RS	0/5	1/5	2/5
			1:480 by vol.	5 drops	0/50	11/50	17/50	31/50	36/50	5 ml.	RS	0/10	0/10	0/10
Guthion 25W 25% Wettable Powder	Chemagro	H_2O Suspension	2 g./gal.	5 drops	0/10	0/10	0/10	0/10	0/10 (82)	—	—	—	—	—

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Product (Alphabetical by Classification)	Manufacturer or Formulator	Use Form	Dilution	Toxicity to Mites[1]						Toxicity to Reptiles[2]				
				Dosage	No. Dead/No. Tested					Dosage	Spec.[4]	No. Dead/No. Tested		
					4hrs	*24hrs*	*48hrs*	*72hrs*	*96hrs*[3]			*24hrs*	*96hrs*	*168hrs*[3]
ORGANIC CHEMICAL PRODUCTS														
Phosphorous Phenyl (carbocyclic) Derivatives:														
Dicapthon, Tech.	Amer. Cyanamid	Ethyl Alcohol Sol.	1:1000 by wt.	5 drops	0/10	4/10	5/10	6/10	7/10	—	—	—	—	—
Methyl Trithion, Tech.	Stauffer	Ethyl Alcohol Sol.	1:1000 by vol.	5 drops	0/10	0/10	0/10	5/10	10/10	—	—	—	—	—
Trithion, Tech.	Stauffer	Ethyl Alcohol Sol.	1:1000 by vol.	5 drops	0/10	0/10	0/10	5/10	10/10	—	—	—	—	—
Repellents:														
Benzyl benzoate	Monsanto	Ethyl Alcohol Sol.	5%	5 drops	2/10	3/10	—	—	—	—	—	—	—	—
Tabutrex, Tech.	Glenn Chemical	Ethyl Alcohol Sol.	3 ml./gal.	5 drops	5/10	6/10	8/10 (41)	—	—	—	—	—	—	—
Delphene, Tech.	Hercules	Ethyl Alcohol Sol.	5%	5 drops	5/10	10/10 (19)	x	x	x	2 ml.	L	5/5 (3)	x	x
			5%	—	—	—	—	—	—	5 ml.	RS	5/5 (1)	x	x
Rodenticide:														
Warfarin	Penick	Dust w/Attaclay X-250	0.025%	3 mg.	2/10	7/10	8/10 (28)	—	—	0.5 g.	L	0/5	0/5	0/5
			0.025%	—	—	—	—	—	—	2 g.	RS	0/5	1/5	3/5
Sulfonates, Sulfides, Sulfones:														
Mitox 40W 40% Wettable Powder	California Chem.	H_2O Suspension	4.5 g./gal.	5 drops	0/10	0/10	1/10	5/10	6/10 (82)	—	—	—	—	—
Orthotran 50W[9] 50% Wettable Powder	California Chem.	H_2O Suspension	4.5 g./gal.	5 drops	0/10	1/10	1/10	1/10	2/10 (82)	—	—	—	—	—
Ovotran W[9] 50% Wettable Powder	Dow	H_2O Suspension	3 g./gal.	5 drops	0/10	6/10	—	—	—	—	—	—	—	—
Sulphenone 50W 50% Wettable Powder	Stauffer	H_2O Suspension	3 g./gal.	5 drops	0/10	0/10	—	—	—	5 ml.	GS	0/2	0/2	0/2
		Dust	None	—	—	—	—	—	—	2 g.	GS	0/2	1/2	2/2 (139)
Tedion 25W 25% Wettable Powder	Niagara (FMC)	H_2O Suspension	4.5 g./gal.	5 drops	0/10	0/10	3/10	5/10	5/10	—	—	—	—	—
Other Organic Chemical Products:														
Ethyl Alcohol	Missouri Solvents	Liquid	95%	5 drops	0/10	0/10	—	—	—	—	—	—	—	—
FOAH Modified 1% Sodium borohydride in saponified red fish oil	Metal Hydrides, Inc.	H_2O Solution	10,000 ppm	5 drops	0/10	2/10	3/10 (28)	—	—	—	—	—	—	—
Methyl salicylate[10] (Oil of wintergreen)	—	Liquid	None	5 drops	5/10	10/10 (21)	x	x	x	—	—	—	—	—

TABLE I. COMPARATIVE ACARICIDAL ACTIVITY AND TOXICITY TO REPTILES OF VARIOUS PRODUCTS SCREENED

Product (Alphabetical by Classification)	Manufacturer or Formulator	Use Form	Dilution	Toxicity to Mites[1]						Toxicity to Reptiles[2]				
				Dosage	No. Dead/No. Tested 4hrs	24hrs	48hrs	72hrs	96hrs[3]	Dosage	Spec.[4]	No. Dead/No. Tested 24hrs	96hrs	168hrs[3]
INORGANIC CHEMICAL PRODUCTS														
Silica Aerogels and Derivatives:														
Dri-Die (SG-67)	Davidson Chem.	Dust	None	3 mg.	0/10	8/10	10/10 (30)	x	x	2 g.	RS	1/5	3/5	4/5
4% Ammonium fluorosilicate	National Biocides		None	20 mg.	16/50	48/50	50/50 (27)	x	x	—	—	—	—	—
			None	10 mg.	21/50	50/50 (9)	x	x	x	—	—	—	—	—
Silikil 4% Ammonium fluorosilicate	United Heckathorn	Dust	None	3 mg.	0/10	2/10	8/10 (28)	—	—	—	—	—	—	—
Silikil D A dense grade of the above.	United Heckathorn	Dust	None	3 mg.	0/10	7/10	9/10 (28)	—	—	—	—	—	—	—
Silikil PY[11] 1.5% Ammonium flurosilicate 0.11% Pyrethrins	United Heckathorn	Dust	None	3 mg.	7/10	10/10 (17)	x	x	x	—	—	—	—	—
Talcs:														
Attaclay	Min. & Chem. Corp. of America	Dust	None	3 mg.	0/10	6/10	10/10 (28)	x	x	—	—	—	—	—
Attaclay X-250	Min. & Chem. Corp. of America	Dust	None	3 mg.	0/10	0/10	0/10 (28)	—	—	1 g.	RS	0/10	0/10	0/10
PROPRIETARY PRODUCTS														
Baume Bengué (contains Methyl salicylate[12], menthol and lanolin)	Thos. Leeming & Co.	Salve	None	Vapor from 3 mg.	0/10	10/10	x	x	x	2 g.	GS	2/2 (2)	x	x
					—	—	—	—	—	2 g.	RS	2/2 (9 minutes)	x	x
El Vampiro Flea Powder 1.250% Piperonyl cyclonene, Tech. 0.125% Pyrethrins 0.625% Rotenone 1.250% other Cubé extractives	J. Strickland & Co., Memphis, Tenn.	Dust	None	3 mg.	0/10	6/10	10/10 (45)	x	x	2 g.	RS	5/5 (5)	x	x
			None	—	—	—	—	—	—	2 g.	GS	2/2 (18)	x	x
Wonder Mite Ball[13]	Pet Accessories, Inc.	Vapor	None	—	1/10	1/10	3/10[14]	—	—	—	—	—	—	—

[1] All controls lived for at least 96 hours. [2] In tests using 5 ring-necked snakes (RS), one of the 5 controls died after 168 hours. [3] The number of hours elapsed at the termination of the test is shown in parentheses when this number is smaller than that shown at the top of the column. — means "no further data or observations"; x means "all animals dead". [4] Under species of reptiles, L—lizard (*Anolis carlinensis*); RS—ring-necked snake (*Diadophis punctatus arnyi*); and GS—garter snake (*Thamnophis radix* or *Thamnophis sirtalis parietalis*). [5] Also see Silikil PY under Inorganic Chemical Products and El Vampiro Flea Powder under Proprietary Products, both of which contain pyrethrins. [6] See El Vampiro Flea Powder under Proprietary Products. [7] Also see Sulphenone 50W under Sulfonates, etc., in the Organic Chemical Products. [8] See "Wonder Mite Ball" under Proprietary Products. [9] A brand of Ovex. [10] A principal ingredient in Baume Bengué; see Proprietary Products. [11] Also see Pyrethrins, Synergized, under Botanicals in Organic Chemical Products. [12] Also see Methyl salicylate under Other Organic Chemical Products. [13] Net contents 1/4 oz. in plastic ball. Contained 3.5% Lindane and 4% other isomers of Benzene hexachloride. [14] One of the mites killed was apparently crushed by the ball.

test and were placed in one-quart glass jars. The acaricides were then applied at the rate of 2 cc. for spray compounds and 0.5 gram for the one dust compound tested against lizards. A moist cloth was secured over the opening of each jar to prevent escape of the lizards, to permit air circulation and to help maintain a reasonably high humidity. Observations were made at one to four hour intervals over the first 100 hours, with gaps of 12 to 15 hours between hours 6 and 18, 32 and 44, 52 and 66 and 75 and 90. Thereafter, checks were made at approximately 24-hour intervals until 168 hours had passed. At each check, the numbers of healthy, affected and dead lizards were recorded.

The first tests on snakes were conducted in essentially the same manner, using groups of five ring-necked snakes and applying the acaricides at the rate of 5 cc. for sprays and 2 grams for dusts. However, this proved to be too drastic, especially with the dust compounds, and several snakes died by strangulation, even in the controls. These were found with their mouths and throats full of the fine, dry dusts. In subsequent tests, one-gallon, wide-mouthed glass jars were used and drinking water was supplied in each jar. Acaricides were applied in the same quantities as before and the tests were conducted on groups of 10 ring-necked snakes per jar and two garter snakes per jar because the latter were in shorter supply. In these experiments observations were made at intervals similar to those used in the lizard tests and all of the snakes survived unharmed in the controls for more than 168 hours. All of the acaricides in the aforementioned tests were applied in the concentrations suggested by their manufacturers or suppliers.

Materials that passed the first two tests, killing samples of 10 mites in less than 24 hours while proving to be harmless to reptiles, were further tested against the mites. In this group of tests the acaricides were applied at one-half the recommended concentration and 50 mites were used in each test. Following this series, four of the more promising acaricides were selected for testing under simulated zoo conditions.

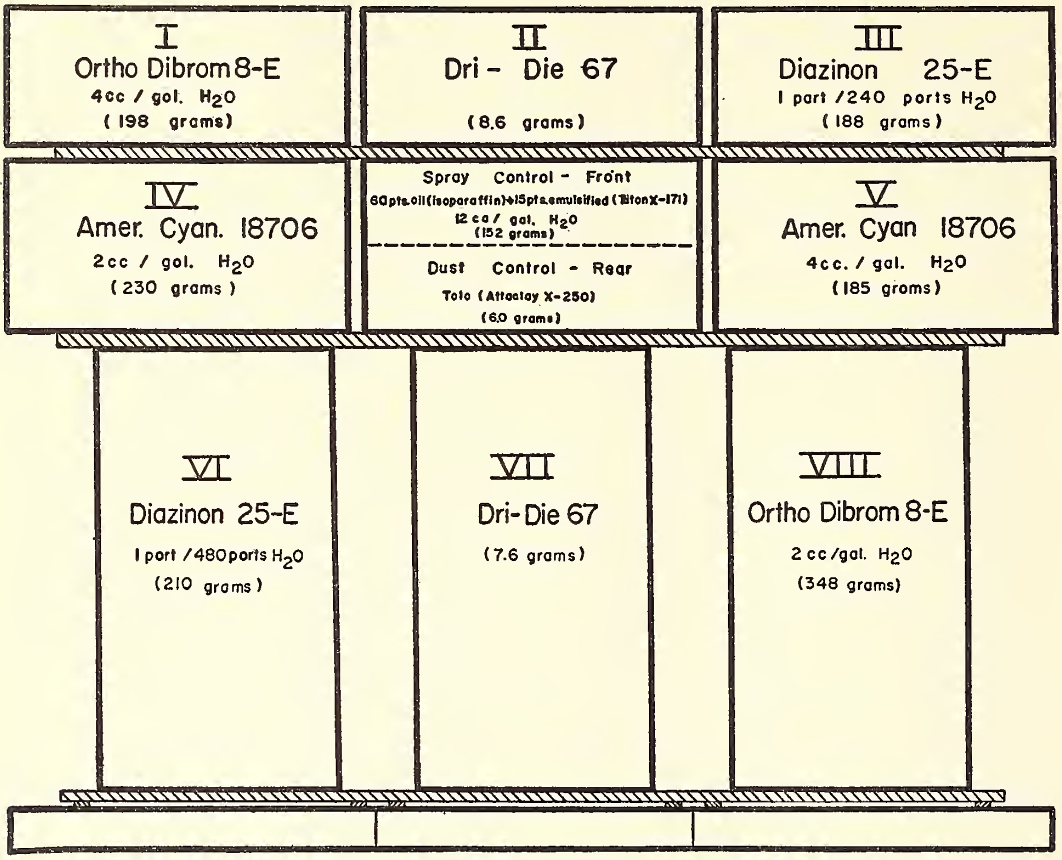
Six small and three larger reptile display cages were obtained from the Swope Park Zoological Gardens in Kansas City. These were constructed of wood, with a screen top and a glass front. The inner two side walls and back were lined with galvanized sheet metal upon which was painted a "natural habitat" scene. The smaller cages measured 24 inches in width, 16 inches in depth, and 11 inches in height. The larger cages were 17 inches wide, 22 inches deep and 29 inches high. The floor of each cage was covered with a mixture of vermiculite and white sand at the rate of approximately 12 to 13 pounds for the small cages and 16 to 17 pounds for the larger ones. Each cage was provided with a water bowl and several natural objects, such as rocks and small tree branches.

One of the smaller cages, which was to serve as the control cage, was divided into front and rear halves by a partition so that both liquid spray and dust controls could be employed.

The cages were arranged in three tiers of three cages each, with the larger cages on the bottom row over some large pans containing water. The water served to raise the humidity in the immediate vicinity of the cages and to prevent the escape of the mites. The control cage was placed in the middle of the second row, in the center of the battery of nine cages (Text-fig. 1). Temperature was maintained at approximately 25° C throughout the experiment but we were unable to maintain constant humidity. Ambient humidity ranged from 48 to 74% R.H., but humidities within the cages and in the microhabitats were probably considerably higher.

Eighty-seven snakes, representing five species, were used in this experiment. These were distributed as evenly as possible, according to size and species, among the cages (Table II). The snakes that were obtained from commercial dealers and from the Swope Park Zoo were already infested with mites. The remaining freshly collected snakes became infested soon after being placed in the colony. All of the snakes appeared to be in good health and most of them were feeding well. All were offered food (mice, earthworms and raw fish) before the start of the experiment. A few refused all food, but no attempt was made to force-feed them. At the start of the experiment all but the smallest of the snakes were well infested with snake mites, but the mite population varied in each cage. Very small snakes do not support significant numbers of mites because their scales are small and overlap closely. Therefore the juvenile garter snakes and the small ring-necked snakes were used to test the safety of the treatment rather than its effectiveness in mite eradication.

Each of four materials was tested at two dosage levels, with each dosage level being tested in a separate cage (Text-fig. 1). The cages were treated individually in another laboratory in order to avoid contamination of any of the other cages in the battery. Liquid compounds were applied with a portable commercial stainless steel pressure sprayer (B & G Model 54-S) and dusts were applied by means of a commercial



Text-fig 1. Arrangement of cages for "field" testing under simulated zoo conditions.

hand-operated insecticide duster (Getz Duster). The equipment was thoroughly washed and dried before each use. The materials were applied with steady, even strokes. First, the inside walls were sprayed (or dusted) from bottom to top, next the top and the floor of the cage were treated and, finally, the acaricide (or control material) was applied to the outside walls of the cage. Every exposed area was covered with the material and cracks, corners, natural objects and other areas in which mites tended to congregate were liberally treated in order to assure that the acaricide penetrated these places. Immediately before the application of the test substances, fresh water was placed in each cage. The formulation was applied around, on and in the water bowl. Snakes were not removed during treatment and were sprayed liberally as the floor of the cage was treated. None of the snakes were handled during the application of the materials.

After the initial treatment the test was allowed to run for eight days. During this time the snakes were given fresh water daily, but none were fed. Observations were made at the start of the experiment and at approximately 24-hour intervals for the first 96 hours and again at 168 and 192 hours. The total operation averaged twelve hours per day.

At the beginning of each observation period each cage and its contents were inspected with aid of a hand lens and a restricted beam flashlight. The snakes were checked but were not handled. Notations were then made as to the apparent presence or absence of a mite infestation and rough estimates as to whether infestations were light, moderate or heavy were made.

Next, a 5 ml. sample of sand was taken from the center and from each of the four corners of the cage. Because in each cage the water bowl occupied one corner and natural objects, such as rocks and branches, occupied another, two of the corner samples in each cage were taken near these. The five sand samples were mixed together and from the mixture a 1 ml. sample was taken for microscopic analysis. This sub-sample was spread out in a 50 mm. plastic petri dish and living and dead mites were counted.

Finally, each snake except the juvenile garter snakes and the ring-necked snakes was thoroughly examined for mites. It was not feasible to actually count every mite on the body of each snake, so a method was used that would permit comparative estimates to be made. The snake was held over a white cloth with one hand of the observer placed immediately behind the head of the snake. The other hand was then passed slowly along the length of the snake from the anterior to the posterior end. Many of the mites would cling to the hands, but most of them dropped onto the cloth. With the help of an assistant, the mites on the hands and on the cloth were counted and recorded. The anal region, chin shields and eye sockets were also checked for mites and the snake was then quickly returned to its cage. The counts were neither exact nor complete, but they provided a reliable means for comparing mite populations in the different cages. After sampling and counting, the mites were placed back into the cage from which they had come in order to reduce the effect that the sampling technique might have on the course of the experiment.

At each observation period the activities and apparent condition of the snakes were also noted.

RESULTS

Of the 45 materials that were tested initially against samples of ten engorged female mites each, eleven killed all of the mites in less than 24 hours (Table I). One of these, Baume Bengué ointment, had been recommended as a potential acaricide because it had been used effectively, due to its repellent action, in confining mites in various laboratory experiments (Strandtmann & Wharton, 1958). The active ingredient in this product is methyl salicylate or "oil of wintergreen," so this was also tested and proved highly effective against the mites. However, when the Bengué ointment was applied by hand to two ring-necked snakes and two garter snakes, these immediately began to writhe and thrash about violently. The two ring-necked snakes were dead in less than ten minutes and the garter snakes died within two hours. Another of the eleven candidates, Silikil PY, is principally a silica aerogel similar to Dri-Die 67, the compound with which Tarshis *(op. cit.)* has obtained such encouraging results. In our initial tests, Silikil PY appeared to be somewhat superior to Dri-Die 67. Silikil PY killed ten mites in 18 hours, while Dri-Die 67 took 30 hours to kill all ten. However, because two other Silikil formulations were less effective than Dri-Die and because of the results obtained by Tarshis with this latter product, we decided to confine further testing of silica aerogels to Dri-Die 67. Nevertheless, Silikil PY showed promise and should be tested more thoroughly. It is interesting to note that most of the remaining formulations among the "most promising 11" are organic phosphorus-containing acaricides (Table I).

Because of the limited numbers of small reptiles available for testing, one other acaricide (Hercules 5727) that had killed ten mites in less than 24 hours and Dylox, which had killed ten mites in 26 hours, were not screened for toxicity

TABLE II: DISTRIBUTION OF SNAKES FOR ACARICIDE TEST UNDER SIMULATED ZOO CONDITIONS.

	Snakes				
Cage	*Elaphe obsoleta obsoleta*	*Natrix sipedon sipedon*	*Thamnophis sauritus sackeni*	*Thamnophis sirtalis parietalis*	*Diadophis punctatus arnyi*
I	1 L*	1 M*, 1 S*	2 M	2 J*	none
II	1 L	1 M, 2 S	2 M	1 S, 3 J	none
III	1 L	2 M	2 M	1 S, 2 J	none
IV	1 L	1 M, 2 S	2 M	1 M, 2 J	none
V	1 L	1 M, 2 S	2 M	2 J	none
VI	1 L	3 M	2 M	2 J	none
VII	1 L	1 M, 2 S	1 M, 1 S	2 J	1 S
VIII	1 L	2 M	1 M, 1 S	2 S, 2 J	none
Spray control	1 L	1 M, 2 S	2 M	1 M, 2 J	none
Dust control	1 L	1 M, 2 S	2 M	1 S, 2 J	1 S

*L = Large = more than 36 inches in length.
M = Medium = 24 to 36 inches.
S = Small = 12 to 24 inches.
J = Juvenile = less than 12 inches.

to reptiles. The remaining seven materials were tested against small lizards and snakes according to the procedures previously described.

Delphene (diethyltoluamide), DDVP and Pyrenone Dust Base all proved to be highly toxic to anoles and ring-necked snakes. Malathion appeared to be safe for the lizards, but killed ring-necked snakes. This compound had been used effectively in snake mite control several years earlier at the Lincoln Park Zoo in Chicago (Camin, unpublished). At that time it was found that dosages as low as 0.01% Malathion emulsifiable in water was effective against the mites and harmless to snakes, if it did not come into contact with the mucous membranes of the latter. However, if even a few drops got into the mouth of a snake, the animal died in a few hours. This was confirmed in the present tests. Adverse effects of Malathion on other vertebrates are not unknown. In commercial production of laboratory mice near Kansas City, it was found that 5% or 2½% Malathion dusts applied to the bedding completely controlled the mouse mite, *Mycoptes musculinus.* Simultaneously, production of marketable young mice was very noticeably reduced in Malathion-treated populations when compared with that of the untreated mite-infested mice (Beran, Cornett & Shuyler, unpublished). Therefore, inasmuch as equally effective and safer materials are now available, Malathion can no longer be recommended for snake mite control.

Of the eleven candidates that killed ten mites in less than 24 hours, only three proved to be harmless to the lizards and small snakes. These were American Cyanamid 18706, Ortho Dibrom 8-E and Diazinon 25-E. It should be pointed out that Hercules 5727 and Dylox also remain as potentially useful compounds in the control of the snake mite, at least until their effects on snakes are known. These should be tested for toxicity to reptiles.

In the initial tests, four additional compounds killed all ten mites in 30-35 hours. These were Delnav, El Vampiro Flea Powder, Dri-Die 67 and one of the formulations of Attaclay. Delnav is another of the organic phosphorus-containing compounds and should be further tested for toxicity to reptiles. El Vampiro Flea Powder, a mixture of piperonyl cyclonene, pyrethrins and rotenone, was recommended to us by an amateur herpetologist who has used this product for several years with good results and no apparent harm to the snakes. We found it to be effective against the mites, but highly toxic to small snakes. Attaclay, a talc that is used as a diluent for some of the dust formulations, probably kills mites by desiccation in much the same manner as the silica aerogels and is probably deserving of further attention. Attaclay X-250, another form of this talc product, killed no mites in the initial tests.

Dri-Die 67, principally a silica aerogel that abrades the epicuticle and adsorbs its lipid waterproofing layer, thus causing death by desiccation, has given somewhat erratic results. Because this compound is not in itself thought to be toxic to the mites, but kills indirectly through desiccation, its effectiveness is probably affected in some degree by the humidity to which the mites are subjected and also by the activity of the mites. In the initial test against ten mites, 3 mg. of Dri-Die 67 killed half of the mites in 20 hours and required 30 hours to kill all ten. In two subsequent tests, using 10 and 20 mg. of Dri-Die 67 against samples of 50 engorged female mites each, the lower concentration killed all the mites in nine hours while the higher concentration required 27 hours to produce 100% mortality. In both cases half the mites were dead after only five hours. While employing Dri-Die 67 for snake mite control in the laboratory at The University of Kansas it had been noted (Camin, unpublished) that while the compound produced rapid desiccation and death in mites that were off the host or crawling about freely on the host, mites that were attached under the scales and feeding appeared to remain attached much longer than usual. While being affected by the acaricide, these mites apparently compensated for the body water lost through dessication by imbibing more blood from the host. More than a week after treatment with Dri-Die 67, although no mites could be found crawling freely about the cage or on the host, fully engorged and living mites were still plentiful under the scales and in the eye sockets of the hosts. It was also noted that while Dri-Die 67 appeared to be harmless to large snakes and even to most small snakes, it apparently caused some desiccation in the reptiles too, because treated snakes drank much more water than did untreated snakes in nearby cages. Generally, the snakes seemed to be able to maintain their water balance and suffered no harm, but some of the smaller snakes died after being treated with Dri-Die 67, apparently due to desiccation. However, because of the excellent results reported by Tarshis *(op. cit.)* and the promising, though erratic results that we had obtained with Dri-Die 67, we decided to continue to test this product.

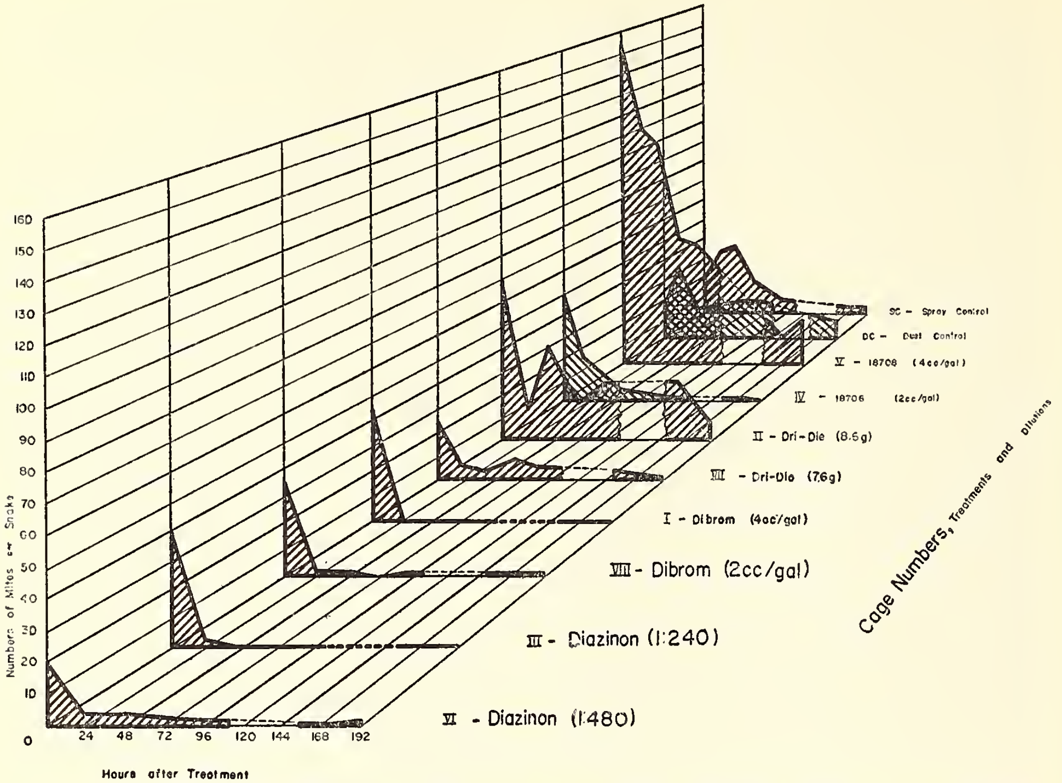
Sulphenone and Warfarin were also recommended to us as potentially effective acaricides. Sulphenone, in aqueous suspension, was harmless to snakes, but it also killed no mites. The producers then suggested that we use it dry and undiluted. This proved to be toxic to snakes and no further tests against mites were conducted. Warfarin, a rodenticide, killed more than half the mites in 48 hours, but failed to kill any more. Further tests showed it to be detrimental to small snakes, so testing was discontinued. Several other materials produced significant mite mortality (Table I), but time did not permit us to test them for toxicity to reptiles.

Dri-Die 67 and three other materials were selected for final "field" testing on mite-infested snakes under simulated zoo conditions. The three candidates, all of which had killed 10 mites in 24 hours and had proved relatively non-toxic to small reptiles, were American Cyanamid 18706, Ortho Dibrom 8-E and Diazinon 25-E. As was done earlier with Dri-Die 67, each of the three formulations was tested at one-half concentration against samples of 50 engorged female mites prior to the "field" test. All produced 90-100% mortality, but acted more slowly than they had at full concentration. American Cyanamid 18706 killed more than half the mites in less than 20 hours. At 24 hours none of the mites were active, but nine were still alive. At 44 hours 100% mortality was achieved. Ortho Dibrom 8-E had killed half of the mites by the end of 30 hours and of the remaining 25, only five were able to move about. By the end of 96 hours only five were still alive and four of these were paralyzed, but two remained alive to the end of the test at 168 hours. Diazinon 25-E required approximately 60 hours to kill half the mites and at 96 hours, the time at which a few of the mites in the controls began to die, fourteen were still living. Four mites survived to the end of the test, but none were moving at that time.

The final experiment, simulating conditions to be found in some zoo collections of reptiles, was purposely designed to make the eradication of snake mites difficult. We believed that if eradication could be accomplished under such circumstances, we would then have a method of control that could be expected to meet with reasonable success even in the most poorly curated of serpentaria. This is most important because such establishments are often the sources of specimens for the most carefully kept collections. Therefore, the control cages were placed in such a manner as to give the mites from these cages free access to the treated cages. It was originally intended that this experiment would be repeated several times with different arrangements of the cages that were treated with the various acaricides, in order that the experiment would not be biased for or against any of the test materials. Unfortunately, time and funds did not permit the repetition of these experiments and the project had to be terminated without being truly completed. Nevertheless, keeping these drawbacks in mind, much valuable data resulted from the one "field" test that was completed.

In an evaluation of the data from the "field" test, several things must be taken into account. Because the lower cages were continuously subjected to re-infestation by mites dropping from the cages above, no two cages in the series were actually subjected to identical conditions. The effects of mites (primarily engorged mites) dropping from the upper to the lower cages were partly counter-balanced by the tendency of unfed mites to climb upwards, thus resulting in some movement of mites from the lower to the upper cages. Considering only downward and upward migration of the mites, then the infestation in cage VII, being directly under the control cages, would be the most difficult to combat. Cages VI and VIII would rank next, cages IV and V would be third, cage II would be fourth, and cages I and III would be fifth or the easiest to control. Another factor to be considered, however, with regard to the probability of re-infestation, is the amount of surface exposed directly to the control cages. From this standpoint cages II, IV, V and VII would be in the worst positions, while cages I, III, VI and VIII would be the most favored. In addition to the problem of re-infestation, the initial mite population size must be taken into account. Because of the facts that populations increase geometrically and the snake mite can complete its life cycle in a very short period of time (13 to 19 days at 25° C), the size of the initial infestation is of paramount importance (Text-fig. 2). From the standpoint of this factor alone, the populations in cages I, III, VIII and the control cages were approximately equivalent. The population in cage VII was lower and that of cage VI was considerably lower. Cages II and IV were more heavily infested and the infestation in cage V was extremely heavy. Taking all these factors into consideration, but giving more weight to the factor of the size of the initial infestation, the mites in cage V should be the most difficult to eradicate and, in order of decreasing difficulty of control, the remaining cages would rank as follows: cage II-2nd; cages IV and VII-3rd; cage VIII-4th; and cages I, III and VI-5th (Text-fig. 1).

The migrating habits of the mites were considered in the placement of each treatment with respect to the spray-dust control cage. We were aware of other promising work with Dri-Die, so we thought it best to give this material a critical test with respect to mite control by placing it above and below the control cages. Less was known about the properties of American Cy-



TEXT-FIG. 2. Comparative effects of four acaricides, at two concentrations each, on snake mite infestations. (No observations were made at 120 or 144 hours).

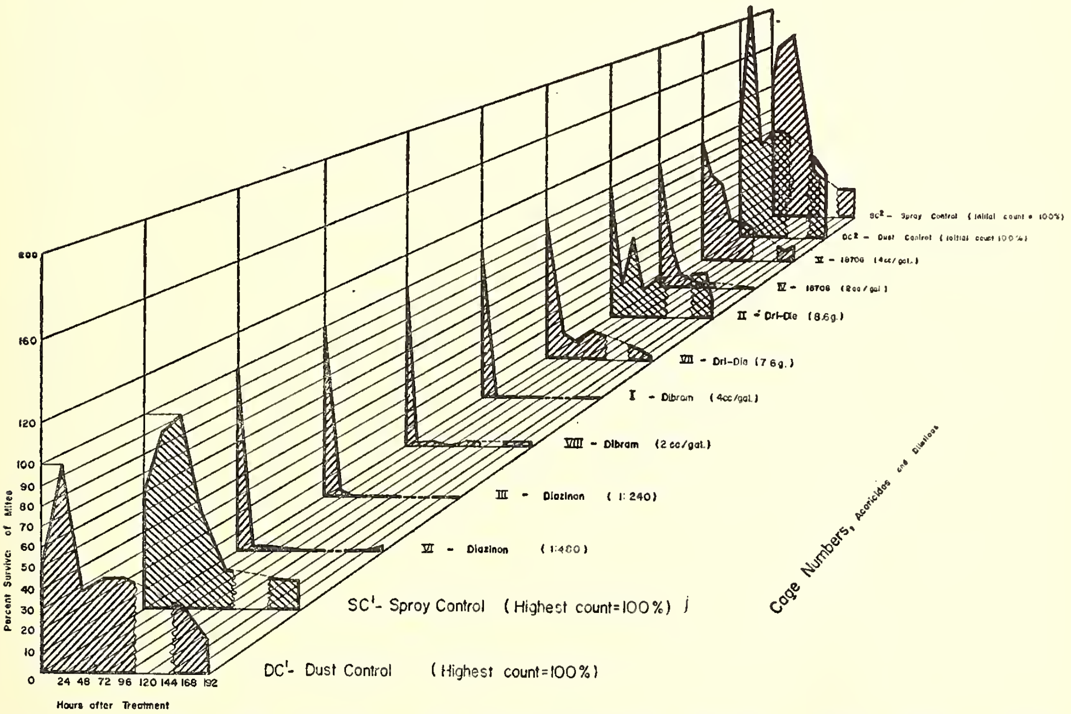
anamid 18706; therefore, it was thought best to test this material in a placement that should be average with respect to migration. In order to test Diazinon, Dibrom and Dri-Die more critically, the lower concentrations were used on the bottom row of cages and heavier concentrations on the upper row of cages.

Merely by random chance, the three lower cages had the lightest infestations of mites, the two upper corner cages had moderate infestations, as did the cage to the left of the control cage, while the cage above the control cage and especially the cage to the right of the control cage had much heavier infestations.

At first examination, it would appear that all four candidates, at all eight concentrations, were highly effective. Judging by means of the method this is commonly used to evaluate the magnitude of the mite problem in reptile collections, that of observing whether or not there are mites crawling freely about on the surfaces of the snakes or on the walls and objects in the cage, all treatments were *apparently* completely successful. In the control cages, mites were readily observable throughout the experiment. In those cages treated with American Cyanamid 18706 (cages IV and V) mites were visible for 72 and 96 hours, respectively, but then disappeared. In cage VI, treated with Diazinon 25-E, mites could no longer be seen after 48 hours, while in all other treated cages the mites were no longer apparent after only 24 hours, with the exception of their temporary reappearance in cage II at 72 hours. Although this method is perhaps reliable in detecting heavy infestations, the fact that it should not be too heavily relied upon is attested to by the remaining data. The sampling of living and dead mites in the sand of the cage is also of some value, but it proved to be too erratic to be very accurate. The only completely reliable method of estimating mite populations on snakes is actually to count the mites on various parts of the body of the snake with the aid of a microscope. This, of course, is not feasible. However, a good indication of the extent of the mite problem can be obtained by sampling the mite populations that are on the snakes by removing them and counting them with the naked eye. It must be noted that the method of sampling that we used, while giving a fairly accurate estimate of the *relative* sizes of the mite populations, does not yield completely accurate estimates of the *abso-*

lute population sizes. This method samples only the mites that are crawling about on the snake, not those that are attached and feeding. Therefore, population estimates are more accurate for the cages that were treated with substances that disturbed the mites and caused them to stop feeding and move about. Thus, the populations of mites in the control cages *apparently* rose at first because the mites were disturbed by the control treatments even though they were not killed by them. Similar disturbance probably occurred in the other eight cages, too. However, in the latter, most of the activated mites, coming in contact with freshly applied acaricide, probably died. Thus, although an initial apparent rise in population probably also occurred in these cages immediately following treatment, the population had decreased significantly by the time 24 hours had passed. It should be noted, however, that although in several cases the experimental treatments appeared to be 100 percent effective, this does not necessarily indicate complete eradication because some mites may have remained under the scales of the hosts, attached and feeding. These would not have been sampled and, in the case of acaricides with little residual activity, these mites could eventually re-establish the infestation.

After sampling and counting the mites from each cage, the mites were placed back into the cage from which they had been removed. It was hoped that this procedure would minimize any direct influence that the sampling technique might have on the course of the experiment. Unfortunately, as our data from the control cages revealed, the sampling was, in itself, fairly effective in reducing the mite populations on the snakes. This fact must be taken into account when evaluating the effects of the various treatments. The sampling technique reduced the populations of mites by approximately 50% in 48 to 72 hours in both controls. If, for purposes of comparison, the highest count obtained is regarded as 100%, then both control populations were reduced to less than 20% by the end of eight days (Text-fig. 3). On the other hand, if it is assumed that the acaricide-treated mite populations would also have shown an initial apparent rise, if mites had not been killed by the treatment, then it is probably more accurate to compare initial samples of acaricide-treated populations with initial control populations. In so doing, the initial count is regarded as 100% and subsequent counts are calculated as percentages of the initial estimate (Text-fig. 3). In this case, the mite



TEXT-FIG. 3. Effects of four acaricides, at two concentrations each, compared by percent survival of mites. (No observations were made at 120 or 144 hours).

populations never fell below 35% in the dust control nor below 25% in the spray control.

American Cyanamid 18706 greatly reduced the mite populations, but its action appeared to be much slower than that of other materials. It did not completely eradicate the mites and those mites that survived the initial application in cage V still caused considerable damage to the snakes. It must be remembered, however, that the cages treated with 18706 (cages IV and V) contained two of the most challenging infestations (Text-fig. 2). If other promising substances did not appear to be available, this acaricide would be well worth using and, therefore, deserves further testing. The fact that better control was accomplished with the lower concentration (cage IV) than with the higher (cage V) emphasizes the importance of considering the initial population size when evaluating the results. One snake died in cage IV and three died in cage V. Because of the fact that the mite populations were extremely high and because the juvenile snakes in both cages survived the experiment, it is suspected that the snakes may have died from exsanguination by the mites rather than the effects of the pesticide. From this experiment alone the possibility of toxic effects on the snakes cannot be completely excluded. However, previous tests had shown this compound to be harmless to small snakes.

One snake in the "Spray Control" cage also died during the course of the experiment. This specimen, too, appeared to have died from loss of blood due to the uncontrolled mite population.

The initial effects of Dri-Die 67 were somewhat more pronounced than those of 18706, but this substance, too, failed to effect eradication and mites were still found crawling on the snakes in both Dri-Die treated cages after 192 hours (Text-figs. 2 & 3). As with 18706, the relative lack of success with Dri-Die 67 in cage II may be due in large part to the high initial infestation. However, comparison with the controls indicates no significant effect in cage II subsequent to the 24-hour observation. After the initial population drop in cage VII, the mite population remained at a fairly low and steady level, but did not disappear. This continuing low infestation may have been due in part to continual re-infestation from the control cage directly above it. Because of these factors, the testing of the mite-eradicating abilities was biased against both Dri-Die 67 and 18706 and should not be construed as proof that these products are necessarily inferior. However, in both Dri-Die treated cages the snakes were obviously dehydrated and consumed considerable quantities of water. The death of one juvenile garter snake in cage VII may have been accidental, but the deaths of six out of ten snakes in cage II must be attributed, directly or indirectly, to the Dri-Die treatment. All of the smaller snakes died and it appeared that dehydration and exsanguination were the primary causes of death. There were indications that the mites, desiccated by Dri-Die, were stimulated to feed continuously. The snakes, despite the fact they they were constantly drinking water, were unable to maintain a proper balance of body fluids and succumbed.

Although the four cages (I, III, VI and VIII) that were treated with Diazinon 25-E or Ortho Dibrom 8-E were favored from the standpoint of position and initial infestation (Text-figs. 1 & 2), the results were so dramatic as to be unquestionably significant. The mite populations in all four cages appeared to be completely or almost completely destroyed in 24 hours. Complete eradication apppeared to have been accomplished in cages I and III by 24 hours, although a singe mite was found in cage I at 168 hours and in cage III at 24 hours. These mites and the very few mites that appeared in cages VI and VIII after the initial drop may have been migrants from the other cages and these may not have had time to succumb. It cannot be concluded that either Diazinon 25-E or Ortho Dibrom 8-E will guarantee eradication of the snake mite, but both compounds appear extremely promising. All of the snakes treated with either of these two acaricides survived the experiment and appeared to be in good health. Subsequent use of Diazinon 25-E in the laboratory at The University of Kansas has proved completely effective against the snake mite in cages of snakes that are kept in the same room and on the same shelf with two untreated cages, which contain thriving experimental colonies of *Ophionyssus natricis*.

Summary and Conclusions

Forty-five materials were each tested initially against samples of ten engorged adult female snake mites. Eleven of the products killed all ten mites in less than 24 hours. Nine of these were then tested for toxicity to small reptiles along with three other slower-acting acaricides that had been recommended by amateur herpetologists. Several of the remaining materials achieved significant mite mortality in varying times greater than 24 hours and many of these deserve further testing (Table I).

Only three of the acaricides that were tested for herpeticidal activity proved to be safe for use on small reptiles. These products, American Cyanamid 18706, Ortho Dibrom 8-E and Diazinon 25-E, were then each tested at two concentrations under simulated zoo conditions. Because Dri-Die 67 had achieved such promising results in other studies (Tarshis, *op. cit.*), it was also included in the "field" test.

The disproportionately large mite populations to which American Cyanamid 18706 was subjected biased the "field" test so that the results of this test were inconclusive with regard to this acaricide. The compound appears to be of potential value in the control of the snake mite and should be further examined. The test was similarly biased against Dri-Die 67, but, while earlier tests showed this aerogel to be highly effective against the snake mite, the results were erratic and there was strong evidence that Dri-Die 67 may sometimes be detrimental to small snakes.

The results of all tests with Ortho Dibrom 8-E and Diazinon 25-E were quite dramatic and very encouraging. Both of these acaricides appear to be capable of eradicating snake mite infestations within 24 to 48 hours without harming the reptile hosts. Both are emulsifiable in water and can be sprayed directly into the cage and on the reptiles, necessitating no special or additional maintenance operations. Ortho Dibrom may be applied in concentrations of 2 to 4 cc. per gallon of water and Diazinon in concentrations of one part of the 25% concentrate in 240 to 480 parts of water. If the first application does not completely eradicate an established infestation, a second application in two to four weeks should accomplish this. After ridding a collection of the original infestation, it should be possible to keep the collection free of mites by instituting a program of rigid quarantine for all incoming reptiles with routine spraying of these with either Dibrom or Diazinon before introducing them into the collection.

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5

Behavior of the Amazon Dolphin, *Inia geoffrensis* (Blainville), in Captivity

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(Plates I-IV; Text-figures 1-5)

INTRODUCTION

THE freshwater or river dolphins of the family Platanistidae appear to be among the more primitive of living cetaceans. The family includes only four Recent species, which have typically relict distributions. The most aberrant of the existing species is the Ganges dolphin, *Platanista gangetica* (Lebeck), which inhabits parts of the Ganges, Indus and Bramaputra Rivers of India. A second species, the white-flag dolphin, *Lipotes vexillifer* Miller, occurs in Tung Ting Lake in central China and the adjacent parts of the Yangtze River some 600 miles above its mouth. Two species are Neotropical. The La Plata dolphin, *Pontoporia blainvillei* (Gervais), is found in the La Plata River and adjoining coastal waters between approximately 30 and 45 degrees South Latitude. It is the only platanistid that is not entirely restricted to fresh water. The Amazon dolphin, *Inia geoffrensis* (Blainville), ranges throughout much of the Amazon River system and also occurs in the Orinoco drainage. *Inia* appears to be most closely allied to *Lipotes.*

The Platanistidae first appear in the Miocene, and 12 genera are known from Miocene, Pliocene and Pleistocene marine deposits in North and South America and Europe. The fossil evidence suggests, therefore, that the platanistids were at one time a widespread and relatively successful marine group. An important factor contributing to their decline may have been competition with the more advanced delphinoid odontocetes, which were well represented by late Miocene and Pliocene times. This apparent replacement of one adaptive level of organization by a more advanced type parallels in an interesting way the replacement of more primitive bony fishes by the teleosts in the Tertiary. A further similarity is the survival of the majority of the archaic bony fish remnants in fresh water habitats.

Although the living platanistids have been the subject of various taxonomic and anatomical studies, almost nothing is known of the details of their natural history. This paper presents observations on the habits and behavior of the Amazon dolphin in captivity. The study was carried out at Silver Springs, Florida, where two specimens captured in the upper Amazon in the vicinity of Leticia, Colombia, were kept alive for over a year. These individuals were the survivors of four specimens captured by an expedition sponsored by Florida's Silver Springs in February and March, 1956, and flown to Florida. Field studies made on *Inia* and the delphinid *Sotalia* during this expedition have previously been published (Layne, 1958).

We made observations on the *Inia* at Silver Springs throughout the entire period of their captivity. The extreme clarity of the water in which they were kept and the manner in which they were maintained afforded ideal opportunities for observations. This was particularly true during the first two months following their capture, when they were kept under more natural conditions and isolated from the general public.

During their early captivity we observed the

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dolphins several times a week for periods ranging from one to five or six hours. Later we visited the dolphins at intervals of from one to several weeks. Usually, we watched the animals from the banks of the channel in which they were initially kept or from the roof of a "submarine" boat moored alongside the pen in the main spring to which they were later transferred. The roof of the boat was some 6 to 8 feet above the water and provided an excellent vantage point from which to observe the activities of the dolphins anywhere in the enclosure, either at or below the surface. The boat also had a deep well with portholes several feet below the water line through which we could make closer observations on the activities of dolphins while they were swimming under water.

ACKNOWLEDGMENTS

We are grateful to the management of Florida's Silver Springs, particularly Mr. William B. Ray and Mr. Ricou Browning, for the opportunity of making this study and for the many courtesies extended to us during our visits to the Springs. Both Messrs. Browning and Ray provided much information that supplemented our own data. Dr. J. C. Dickinson, Jr., Florida State Museum, University of Florida, accompanied us on several occasions and made a series of 16 mm. movies that have been of much value in analyzing certain aspects of the behavior of the dolphins. Mr. E. Ross Allen of the Ross Allen Reptile Institute at Silver Springs permitted us to study a movie made by persons on his staff, and Dr. Wilfred T. Neill and Mr. Warren Prince of the same organization supplied us with notes of their own observations on the *Inia*. Dr. William Schevill, Woods Hole Oceanographic Institution, and Barbara Lawrence, Museum of Comparative Zoology, Harvard University, aided in making observations on two occasions and very kindly made available their recordings of underwater sounds of *Inia*. Mr. F. G. Wood, Jr., Marineland Research Laboratory, Marineland, Florida; Dr. Kenneth Backhouse, Charing Cross Hospital Medical School, London; and Dr. Nicholas E. Collias, Dr. Kenneth S. Norris and Mrs. Melba C. Caldwell, all of the University of California, Los Angeles, read the manuscript and offered many valuable comments. Dr. Allan McIntosh, U.S.D.A., Animal Disease and Parasite Research Division, Beltsville, Maryland, provided identifications of parasites, and Dr. James E. Böhlke, Academy of Natural Sciences of Philadelphia, identified fish remains found in the stomach of a wild-caught specimen. We express our sincere thanks to all of the above for their respective contributions.

CAPTURE AND TRANSPORT OF SPECIMENS

Various means were employed by the Silver Springs party in an attempt to capture *Inia*. A net approximately 100 yards long, 10 feet deep and with 2-inch meshes, was used on several occasions in an effort to trap dolphins as they moved along the border of flooded jungles or pursued fish off points of land. The results were unsuccessful, as high waters made it difficult to confine the animals and individuals seemed to easily detect the presence of the net, possibly through echolocation, and avoided it without difficulty. One attempt was made to stun the animals by means of an underwater explosion in the hope that they could be more easily captured with the net or harpooned in a non-vital part of the body. The site selected for this attempt was at the entrance of a channel leading from the Amazon to some lakes about a mile north of the town of Leticia. This channel was regularly used by dolphins in moving back and forth between the lakes and the main river. The channel was blocked with the net at a point about 100 yards from its entrance to the river. As a dolphin approached the net on its way through the channel a single stick charge of dynamite was exploded underwater within a few feet of the animal. Although the *Inia* appeared to have been stunned by the blast, it escaped by swimming over the top of the net at a point between floats where the net sagged several feet below the surface.

It was the general opinion of the inhabitants of the region that capturing *Inia* alive would have been considerably easier during the dry season, when the animals could be trapped in shallow coves or narrow channels. They reported that *Inia* were not infrequently captured in fish traps during times of low waters.

Specimens were finally obtained by Indian fishermen who waited quietly concealed at the edge of the flooded jungles along watercourses frequented by the dolphins and harpooned animals that swam within range. On March 4, a fisherman harpooned a large male *Inia* about 7 feet in length and weighing approximately 300 pounds. The animal swam off and was later found about a mile away with the float and harpoon line entangled in some bushes along the edge of the flooded forest. It was hauled into a 16-foot boat and taken to the town, where it was placed in a large concrete fish pond. The following day a small female and male were secured near the spot where the first specimen was taken. All three animals were struck in the dorsal musculature at a point above and just behind the pelvis. The small male appeared to have been more severely injured than the others, as he swam more slowly and laboriously when released in the pool.

The dolphins were loaded on board a plane on the morning of March 6, each receiving an injection of penicillin at the time. The two small individuals were placed in a small dugout canoe containing a few inches of water and covered with wet cloths. The large male was suspended from the roof of the cabin in a canvas sling and covered with a wet sleeping bag.

The attendants reported that all three animals struggled considerably en route to Florida. At one point in the flight, when the plane was at an altitude of 18,000 feet, the small male grew very weak, until finally no heartbeat could be detected. He was revived by artificial respiration and the administration of oxygen by means of an oxygen mask placed over the blowhole. The small female died during the flight. While dissecting this animal later, Dr. K. M. Backhouse found an abcess in the musculature of the neck, which might have been a secondary infection resulting from the harpoon wound and, together with exhaustion, a contributing factor in the death of the animal.

The plane bearing the dolphins arrived at the Ocala, Florida, airfield on the morning of March 7. The two surviving dolphins were transported by truck to Silver Springs and placed in a side channel off the main river. As the animals were being lowered into the water, the large male struggled free of his wrappings and swam off in a normal manner. The small male was noticeably weak and had to be supported in the water in order to breathe. He seemed to gain strength after being in the water a few minutes and began to swim weakly about without support, although he continually ran up into the shallows along the edge of the channel. Within an hour, he had weakened again and despite administration of oxygen through the blowhole and injections of antibiotics and B vitamins went into convulsions and died.

A fourth *Inia,* a small male, captured near Leticia in a manner similar to that of the others, was flown to Tarpon Zoo, Tarpon Springs, Florida, on March 11. He was held in a pool overnight at Tarpon Springs and transported to Silver Springs, a distance of about 100 miles, by car the following day. He appeared to be in good condition upon arrival and behaved normally when introduced into the channel with the larger male.

As is the general case with marine dolphins (Kritzler, 1952), none of the *Inia* offered any resistance to being handled when once out of water. However, the large male struggled considerably when he was being captured for removal from the fish pond in Leticia. Several times a man attempting to hang onto the dolphin's tail stock was thrown completely clear of the water.

We have knowledge of a number of other *Inia* brought alive to this country subsequent to the four mentioned above. Nine of these specimens were received by the Tarpon Zoo. According to Trudi Jerkins, co-owner of that establishment, seven young and adult inias were successfully flown alive from Leticia to Tarpon Springs in 1956, a short time after the Silver Springs expedition had obtained theirs. These inias were also taken with harpoon and lived in captivity for varying periods of time, the maximum being about a year. The animals were kept in a fenced enclosure about 50 by 25 yards in a small pond. They were fed artificially but may have captured some natural food from time to time. The animals all died within a period of a few days. Death was suspected to have been caused by the accumulation of an organic insecticide in the pond as the result of aerial spraying of nearby citrus groves.

Another specimen obtained by the Tarpon Zoo was an old female captured at Leticia on March 18, 1959, and flown to Tarpon Springs a week later. Although the *Inia* appeared to be in relatively good condition on arrival, she died shortly afterwards from causes unknown. Starvation seemed to be ruled out as a possible factor in the death of this animal as it was reported to have fed well during the week it was held in a pond in Leticia. Tarpon Zoo also supplied a female *Inia* to the Forth Worth Zoological Park, Ft. Worth, Texas, in the spring of 1962. The Fort Worth Zoological Park also obtained a second female in July, 1962, from the Gulf Fish Hatchery, Inc., a Florida firm dealing in exotic fishes. This specimen was reported to have been taken by net in the upper Amazon.

In 1963, several additional live *Inia* were also brought from the upper Amazon for display at a tourist attraction at Homosassa Springs, Florida.

Upon their arrival at Silver Springs, the *Inia* were placed in a small side channel connected to the main spring and river. This area was well removed from human activity, thus the dolphins were relatively little disturbed during the early period of their captivity.

The section of the channel in which the dolphins were confined by means of blocking fences was about 300 yards long and varied from approximately 20 to 25 feet in width. The banks were generally wooded and some portions of the channel were shaded during most of the day. The remains of an old foot bridge were located nearly midway between the two fences, and pools 4 or 5 feet in depth alternated with very shallow areas along the length of the stream. A prominent, shallow sand bar was situated a short distance below the bridge. Several weeks after the inias were placed in the channel, the bottom was dredged to provide a deeper passage between pools. The pools most utilized by the dolphins were located at the upstream fence, just above and below the bridge, and about 30 yards downstream from the bridge.

The bottom of the stream was sandy, although overlain with silt in most areas. The sides were generally more silty than the center. Beds of eel grass *(Vallisneria)* were frequent, and waterlogged sticks, leaves and other debris were scattered about over the bottom. There was a moderate, steady current in the channel at all times, and water temperatures approximated 70 degrees F., with little seasonal variation. As in the main spring area and river, the water of the channel was exceptionally clear.

In June, 1956, the dolphins were moved from the channel to a large enclosure in the main spring area. This pen had dimensions of about 75 by 40 feet. Its depth ranged from about 5 feet near the bank to a maximum of approximately 10 feet in the deepest portion. The bottom was sandy and partially covered with large patches of eel grass. Shortly after the inias were introduced into the enclosure a mat of water hyacinths *(Eichornia)* was spread over about half of the surface to provide some shade for the animals. During the winter of 1956, the large male became increasingly aggressive toward the smaller individual and it became necessary to separate the animals in the enclosure by means of a fence. This arrangement was maintained until the death of the dolphins the following spring.

Conditions under which observations could be made were somewhat less satisfactory in the main spring than in the channel. There was a much greater disturbance level in the main spring. A bathing beach was situated adjacent to the pen, and numerous power boats passed within a few yards during the daylight hours.

HEALTH

The dolphins appeared to acclimate well to the conditions of captivity. The harpoon wounds healed without complications, and outwardly the animals remained in generally good health until just preceding their deaths the following year.

Shortly after they arrived the dolphins developed fuzzy yellowish patches, apparently a fungus growth, on the flukes, caudal peduncle and flippers. Smaller patches were also present on the snout of the small male and over one eye of the adult. The patches became less noticeable in about a week and soon disappeared entirely.

From time to time abrasions were noted on the snout and leading edges of the dorsal fin, flippers and flukes. These wounds were apparently caused by the dolphins rubbing against objects in the water, an activity in which they frequently engaged.

Both individuals also suffered from cracking of the skin, which was first observed on April 20. This condition was most severe on the dorsum between the blowhole and origin of the dorsal fin and appeared to be the result of sunburn, since the animals at this time were still being kept in the relatively shallow and extremely clear water of the channel and spent much of their time just beneath the surface. The back of the larger male was more severely affected than that of the smaller individual, whose skin was more heavily pigmented and thus probably better protected from the sun's rays. The area around the blowhole, which was well pigmented in both individuals, was not injured. It has been suggested (Layne, 1958) that the persistence of pigment around the blowhole, even in the fully grown, pale-colored adult *Inia,* may be an adaptation for preventing sunburn of that portion of the head regularly exposed above water in the course of breathing. In the natural habitat, the rest of the dolphin's body is probably protected from the sun by the general turbidity of the water. When the inias were transferred from the channel to the deeper water of the pen and a cover of water hyacinths was provided, the condition of the skin improved rapidly.

Both dolphins grew progressively darker during the period of their captivity. This increase in skin pigmentation was probably a response to the greater light intensity in the clear water in which the animals were kept. Similar environmentally-induced changes in coloration apparently also occur under natural conditions, as Jardine (1837) states that inias inhabiting rivers are paler than those in lakes. This trend is probably associated with the usually more turbid water conditions in rivers as compared to lakes.

The dolphins occasionally suffered from respiratory ailments. One such case occurred in the small male a few days after reaching Silver Springs. For a day or so he would give loud snorts when surfacing to breathe. Each snort would be accompanied by a discharge of globules of mucous-like material from the blowhole. The material, which would be expelled to distances of 5 or 6 feet, resembled the nasal discharge of humans suffering from a cold, being yellowish-white, highly viscous and possessing a faint odor. The captives also sometimes uttered similar snorts under circumstances which suggested that the sounds might have some communicatory function (see p. 102).

The small male died in March, 1957, after a year in captivity. On March 23 he appeared to be experiencing respiratory difficulty. The narial passages seemed to be clogged, although no discharge of material was noted at expiration. The dolphin also listed to one side when he came to the surface for air. He had eaten little or nothing for several days. The animal was captured with a net and given injections of penicillin and adrenalin intramuscularly. The following day the dolphin's condition had worsened. Although he seemed to have less difficulty in breathing than the day before, he swam sharply heeled over on the right side and was extremely bloated. As a result of the latter condition he had difficulty in submerging and remaining under water and would pop to the surface like a cork as soon as he ceased swimming movements. At this time he was removed to a stock watering tank where he could be more easily observed and cared for.

Despite administration of antibiotics and other treatment, the dolphin showed no improvement, and by the evening of March 28 there appeared to be no hope for recovery. The dolphin was transported by car to the J. Hillis Miller Health Center at the University of Florida where it was killed by over-anesthetization with ether.

An autopsy revealed several pieces of water-soaked wood in the stomach, congestion in the lungs, and an extensive hemorrhage in the brain, all of which might have contributed to the death of the animal. The hemorrhage in the brain involved virtually the entire right cerebral hemisphere and extended to the midbrain. Flukes, *Hunterotrema caballeroi,* were found in the air passages of the lungs, and later microscopic examination of sections revealed the presence of trematode eggs in the brain.

Additional records of endoparasites have been obtained from two other *Inia* that we have handled. Numerous round worms, *Anisakis insignis,* were present in the stomach of the young female that died enroute during the initial shipment of the dolphins to Florida. Specimens of the same species were also obtained from the stomach of the old female purchased from Tarpon Zoo in March, 1959.

The large male was found dead on the morning of May 10, 1957, after 15 months in captivity. Although he had ceased feeding for a short interval a week or so before, he had otherwise behaved in a normal manner until the time of his death. According to Dr. K. M. Backhouse, who later dissected this animal, death was apparently due to severe bronchial pneumonia involving both lungs.

MISCELLANEOUS PHYSIOLOGICAL DATA

A few physiological data were gathered incidental to other observations. Heart rate was measured on two occasions. An average of 60 beats per minute was recorded for the large male before he was removed from the plane after the trip from Leticia. The intervals between breaths during the period over which the heart rate was determined averaged 63 seconds, with extremes of 18 and 107 seconds. The heart rate of the small male shortly before death averaged 96 per minute, with extremes of 90 and 104. At the time of measurement the dolphin had been out of water for approximately two hours, although it had been covered most of the time with moist blankets. In view of the animal's weakened condition and other factors, it may be assumed that the heart rate recorded on this occasion is well above normal.

A rectal temperature of 97.4° F was obtained for the small male while he was being held in the stock watering tank for examination and treatment. The water temperature was 69° F. Another measurement was made when the animal arrived at Gainesville following a 40-mile trip from Silver Springs by automobile. The rectal temperature on this occasion was 96.9° F. The dolphin had been uncovered for several minutes before the temperature was taken and its skin was fairly dry. In both measurements the thermometer was inserted to a distance of only about 6 inches, thus the temperatures obtained may not have represented actual core values. They do, however, fall well within the range of cetacean temperatures cited by Kellogg (1928) and Wislocki (1933).

The small dolphin died much more rapidly under ether anesthesia than expected. As soon as the ether cone was placed over the blowhole, the animal clamped the lips of the blowhole tightly together and refused to take a breath for an abnormally long interval. The tempo of the dolphin's breathing became more rapid and regular after several breaths of ether and then it abruptly died. Lilly (1958) suggests that the breathing of cetaceans, as an adaptation to an aquatic habitus, is predominantly under voluntary control, which makes anesthesia difficult because the animal ceases to breathe as soon as it looses consciousness. It is not certain that the rapid death of the *Inia* when given ether is explainable on this basis. The transition from irregular to rhythmic breathing observed might have indicated a shift from voluntary to involuntary regulation and death may actually have been due to the specific effect of ether.

Weights of certain organs of the small male, whose total length was 1,877 mm., were as follows: liver, 1,800 gms.; heart, 275 gms.; kidneys (both), 300 gms.; spleen, 23 gms.; lungs, 1,450 gms.; adrenals, 9 gms.; and thymus, 75 gms. The total length of the intestine (large and small together) was 105 feet. The brain weighed 525 gms., giving a brain weight/body length index of 88, a value markedly lower than those given for other cetaceans (Lilly, 1958; Kojima, 1951; Jansen 1952). A red blood cell count of 3,671,000 per mm^3 was calculated by Elizabeth S. Wing from a sample obtained from the body cavity of the small male during the autopsy. Unfortunately, body weight was not recorded for this specimen.

General Behavior and Activity

During the first week or so in captivity the dolphins confined their movements to the part of the stream above the large sand bar mentioned previously. Neither animal was observed to cross the bar and explore the section of the stream below until a channel was dredged through it. Thereafter, the dolphins occasionally ventured into the lower part of the stream, although they still spent most of their time in the section above the sand bar. When crossing the bar, they always remained in the deepest part of the channel.

The dolphins tended to restrict their activity to a certain part of the channel for a time and then shift to another. They would usually remain in a given pool for some time, swimming in slow irregular circles, often following the same path and rising to breathe in nearly the same spot each time, and then make a leisurely excursion up or downstream to another pool. Sometimes they remained in the second pool for an extended period, but more often returned in a short time to the original one to continue idly circling about. While swimming they would often probe the bottom with their long beak, and when in the pool next to the bridge they would frequently thrust the break into the crevices between the logs. The dolphins frequently settled on the bottom.

The animals were somewhat more active in the enclosure in the main spring area. They were observed resting on the bottom only once and showed a lesser tendency to confine their activity to a particular portion of the enclosure for any length of time, although they would sometimes follow the same general path in swimming and surfacing for air for an interval of several minutes. The generally unoriented pattern of movements of the inias in the enclosure appears similar to that of a captive female bottlenosed dolphin studied by Schevill & Lawrence (1956).

Two observations were made that indicated a tendency of *Inia* to become habituated to a particular area. This was first demonstrated when the dolphins were moved from the channel to new quarters in the main spring enclosure. The upstream fence was removed with the intention of herding the animals up the channel to the new pen. However, it proved extremely difficult to drive them beyond the former limits of the fence. The second example of reticence to abandon familiar surroundings occurred when the fence that had been erected across the main spring enclosure to separate the two animals was lowered after the death of the small male. Although seen to explore the opening from time to time, the surviving animal did not venture into the other side for at least five days.

Wild *Inia* appear to exhibit positive rheotaxis (Layne, 1958), and the captives reacted similarly to even the relatively slight current in the channel. When the large male was first released into the channel he circled about nearby. When disturbed by a human swimmer he swam rapidly and unhesitatingly upsteam until stopped by the fence. Since he had no knowledge of the stream at this time, his behavior seems to indicate a response to the current. Both inias showed a definite predilection for the upstream end of the channel, spending much of their time in the pool just below the fence. They would orient themselves into the current and maintain their position with slow strokes of the flukes. Whenever they rested on the bottom they invariably headed upstream. When alarmed anywhere in the channel, the dolphins would usually swim rapidly to the upstream fence. If frightened while swimming downstream they would often continue in the same direction with increased speed for a few feet but then nearly always turned and headed back upstream. If a human being in the water positioned himself between one of the dolphins and the upper fence the animal would become visibly agitated. It would finally rush past, swinging as far out to the side opposite the intruder as the width of the channel would permit, and take refuge in the upper end. Captive *Tursiops* and *Globicephala* exhibit a similar tendency to swim into a current and, at least in the case of the latter, may derive pleasure from the flow of the water over the body (McBride & Kritzler, 1951; Kritzler, 1952).

One of the commonly observed activities of the dolphins was scratching or rubbing. Although they were seen to scratch or rub the body against the bottom or projecting objects as they swam about throughout the period of their confinement, they engaged in extensive periods of such activity for several weeks during the early period of captivity. The cause of the increased scratching during this interval may have been an irritation from what appeared to be a fungal growth on parts of the body, since a reduction in the frequency of scratching behavior coincided with the disappearance of the growth on the skin. On March 16 both animals were observed doing slow rolls while swimming slowly along and rubbing the dorsum and upper sides along the bottom. A week later they were found in the pool below the bridge, where they continually circled and scratched on a stick about a foot long that was sticking up out of the bottom. They would approach the stick swimming either on the back or upright and scrape the body with force sufficient to push the skin into prominent folds. Later they transferred their activity to the next pool downstream, where they spent approximately half an hour scraping their backs on the bottom. They would swim upside down for almost the entire interval between breaths and wriggle from side to side as they dragged their backs over the sand. The dolphins cleaned off a patch of sand about 15 feet in diameter by their actions. They next moved back to the original pool and continued scratching on the same stick as before.

The dolphins were again observed to be engaged in scratching and wallowing activity for long periods of time in the same pools on March 31. They also intentionally rubbed against projecting sticks in their sallies up and down the channel between the pools. A similar tendency to rub and scratch, even to the point of causing abrasions in some instances, has been reported for bottle-nosed dolphins and pilot whales in captivity (Townsend, 1914; Kritzler, 1949; McBride & Kritzler, 1951; Tavolga & Essapian, 1957).

The captive *Inia* exhibited no definite diel activity cycle. On the several occasions that they were observed at night, their general behavior and movements were no different from those seen during the daylight hours. They apparently slept for short intervals throughout the day. While in the channel, they would often settle to the bottom and lie motionless except for slight movements of the flukes. They invariably oriented with the head into the current and usually selected a place where the current was strongest. It is possible that this behavior represented sleep, since the animals would sometimes rise slowly to the surface, breathe, then sink back to the bottom again. The trip to and from the surface was accomplished by little, if any, forward movement and was probably accomplished largely by altering the volume of air in the lungs through muscle contraction or relaxation, although flipper movement may also have contributed. Only on one occasion was an apparently sleeping individual observed in the enclosure in the main spring. In this instance, the large male was seen floating just off the bottom with his body forming an S-curve and only the tail in contact with the substrate. According to the observer, the dolphin remained in this position for some time. Both *Inia* occasionally yawned while swimming beneath the surface, and there seemed to be a general tendency for an increased frequency of yawning in the late afternoon. McBride & Kritzler (1951) noted similar behavior in a pregnant female *Tursiops* near parturition.

LOCOMOTION

As in the wild (Layne, 1958), the Amazon dolphins at Silver Springs normally swam at a very slow speed, averaging only between 1 and 2 m.p.h. The only times they moved with greater rapidity were when they were alarmed or feeding. On two occasions we were able to time individuals with a stopwatch over a measured course after they had been startled into swimming at what probably represented nearly maximum speed. The speeds attained in these instances were only 8 and 10 m.p.h. Wild *Inia* seen swimming under circumstances that suggested they were exerting themselves to their limits appeared to be traveling at about the same speed. A bottle-nose dolphin kept by Schevill & Lawrence (1953) in an enclosure with murky water usually swam at a speed of about 4.5 m.p.h. Free-swimming marine dolphins, however, often travel much faster, speeds ranging from about 12 to 34 m.p.h. having been reported (Petersen, 1925; Gray, 1936; Moore, 1953). On the basis of these data, the Amazon dolphin appears to be an exceptionally slow swimmer.

Jardine (1837) has also noted that *Inia* is neither as agile nor as powerful as marine dolphins and that *Platanista,* too, is generally slow and sluggish in its swimming habits, although it may move swiftly when pursuing fish. The apparently marked differences in swimming speeds between the platanistids and marine dolphins are correlated with the morphological features of the two groups. The long beak and well-developed, flexible neck of *Inia* and other platanistids would appear to offer a severe impediment to rapid swimming because of the difficulty in preventing lateral displacements of the head as the animal moves swiftly through the water. The reduction of the rostrum together with the shortening and fusion of the cervical vertebrae in the advanced marine dolphins are probably among the important adaptive trends accompanying the development of fast swimming habits, an ability which may have contributed significantly to the eventual dominance of the delphinids over the platanistids in late Tertiary seas. The relatively large flukes and flippers of *Inia* also appear to be correlated with its slow swimming habits, since in a slow moving dolphin larger control surfaces are probably necessary to maintain maneuverability and stability. Howell (1930) has stated that the dorsal fin is less effective in maintaining equilibrium in cetaceans than in fishes, the flippers of the former probably being more important in this connection. Thus, the relatively poor development of the dorsal fin of *Inia* may be compensated for by the large size of the flippers (Pl. I, Fig. 1). Another distinctive feature perhaps associated with the mode of locomotion of *Inia* is the marked flexibility of the flippers as compared to those of fast-swimming marine dolphins in which they serve mainly as hydroplanes. On several occasions we observed the captive inias propelling themselves very slowly forward by means of a rowing-like action of the flippers, and an individual rising to blow with little forward momentum would aid its ascent with movements of the flippers.

When the captive *Inia* were swimming at the normally slow cruising rate, the flukes were moved through a vertical angle of approximately 20 degrees. Although the entire body pitched up and down with the strokes to some extent, the principal movement was confined to the tail stock and flukes. The point upon which the rear part of the body pivoted during swimming movements was in the approximate region of the pelvic girdle, as is characteristic of cetaceans in general (Slijper, 1961). The stroke rate of the flukes in slow swimming was about 1 every 2 seconds. On occasion, when the dolphins were swimming slowly, a distinct lateral component was added to the vertical motion of the tail stock and flukes, producing a somewhat oblique stroke. An apparently similar stroking action of the tail has been reported in other cetaceans (Beddard, 1900; Petersen, 1925; Kellogg, 1928).

Ordinarily the dolphins swam just below the surface, with the flippers held nearly horizontal and fairly close to the body. Slight adjustments in their position were made as the dolphins swam along, but they were normally not moved extensively when an animal was proceeding on a direct course. When the dolphins swam near the bottom, the flippers were sometimes lowered and allowed to trail gently along the bottom. Turning was accomplished by throwing the body into a curve and through obvious employment of the flippers. Usually the inboard flipper was held more or less vertical while the outboard one was held more laterally and utilized in a rowing manner to help in the turn. The fish-tailing movements of the caudal peduncle seemed to be exaggerated when the animals were turning.

A particularly striking characteristic of the swimming of *Inia* is the pronounced mobility of the head. When the dolphins were progressing at the characteristically leisurely cruising pace, the head was in more or less constant motion in both horizontal and vertical planes. The head was generally swung from side to side through an angle of about 45 degrees, but was moved to a lesser extent in the vertical plane. Often the dolphins turned the head 90 degrees or more to the axis of the body in order to examine an object to the side or beneath them. On several occasions they were seen to reach back beneath themselves to pick up a fish or to prod an object on the bottom as they passed over it. At such times the skin on the throat was thrown into conspicuous folds. The flexibility of the head in an upward direction seemed to be more restricted than either laterally or ventrally.

The increased power for fast swimming appeared to come chiefly from a greater stroke rate and to a lesser extent from an actual increase in the amplitude of the individual strokes. The pitching movements of the body were more pronounced than in slow swimming, and the head was not moved so extensively. A dolphin swimming just slightly faster than the normal speed moved the flukes about 1 stroke per second; whereas at moderately fast, but not maximum, speeds the stroke rate was increased to approximately 2 per second. When braking after a spurt of speed, the dolphins arched the flukes and caudal peduncle strongly downward.

A frequent maneuver performed by the dolphins was the barrel-roll. This was usually done as they swam slowly along and without interruption of their progress. Occasionally one would perform this feat from a position of rest on the bottom, simply rising off the bottom with no preliminary forward movement, rolling, and then moving slowly ahead. Captive *Tursiops* also engage in this behavior (McBride, 1940).

The dolphins often swam upside down. They would remain in this position from 2 or 3 seconds to the entire interval between breaths and often progressed more than 50 feet in this manner. When swimming upside down they seemed to perform the same movements as in normal swimming and to have about equal control over their speed and direction. In observing this behavior, however, we gained the impression that the use of the flippers was more exaggerated than in normal swimming. One individual was seen swimming on its back while carrying a stick in its mouth with its head bent at an angle of nearly 90 degrees. Another individual was observed to masturbate while in this position. Captive *Tursiops* also swim on their backs; Townsend (1914) believed that there was more lateral action of the tail in this position than in normal swimming. The latter may also have been true with *Inia*.

When swimming slowly, the dolphins would often probe the bottom or clumps of eel grass with the beak in a slow and deliberate manner. Ordinarily they kept to the deeper portion of the channel. Only once was one seen to move into shallow water. In this case, the small male, in apparent avoidance of the larger one, swam on its back into a bed of eel grass in water a foot or so deep, then rolled over, coming to rest on the bottom with the back out of water. It stayed in this position for a moment and then with powerful lunging strokes made its way back into deeper water. Sanderson (1956) states that *Inia* utilizes the flippers in making its way out of water across mud flats. The behavior just described is as close to that described by Sanderson as we have observed, and in this case the flippers did not seem to be employed to any great extent in moving out of the shallow water, although admittedly we were unable to observe the event in detail. The captive *Inia* we have observed out of water have been helpless and were apparently unable to move by the use of the flippers. Although the flippers may be of limited use in moving through shallow water, we are highly skeptical that they could function out of water in the manner described by Sanderson.

Only once during our observations of the dolphins did we see the flukes break the water during swimming. In this instance, both animals were observed to bring their backs high out of the water and give vigorous slaps with the flukes on the water surface. Our impression of this behavior at the time was that the action was purposefully performed, possibly as an act of communication or play. Dr. Kenneth Backhouse told us of observing the leader of a school of *Delphinus* leap into the air and slap the water with the flukes as the school passed a ship some 150 yards away. Captive *Tursiops* also indulge in this behavior (Townsend, 1914; Lawrence & Schevill, 1954; McBride & Hebb, 1948; McBride & Kritzler, 1951), and it has been reported in wild *Tursiops gilli* and other delphinids by Norris & Prescott (1961).

Although wild *Inia* were occasionally seen to leap out of the water (Layne, 1958), this behavior was not observed in the captives, probably because of limitations of space or water depth. Marine dolphins jump frequently under natural conditions, and similar behavior has been reported in captivity (Townsend, 1914; Lawrence & Schevill, 1954). The frequency of spontaneous leaping in the wild and captivity, together with the prodigious feats of jumping performed by trained *Tursiops* and *Lagenorhynchus,* provide further indication of the greater speed, power and agility of the marine dolphins in comparison to the platanistids.

During the early period of captivity, the two dolphins frequently swam about in very close association. The young male was invariably the one to initiate this activity. He would swim up to the larger individual and position himself close beside it, with his head at about the level of the adult's flipper (Pl. I, Fig. 2). The sides of the bodies, flukes, or flippers were sometimes in actual contact as the pair swam about, often surfacing to breathe simultaneously. Although our notes are not specific on the point, we are under the impression that the smaller individual did not appear to be showing typical swimming movements at such times. Although there was some movement of the flukes, this may have been at least partly passive. This swimming behavior appears to resemble that type of assisted locomotion in cetaceans termed echelon-formation swimming by Kelly (1959) and which appears to be widespread among delphinids (Norris & Prescott, 1961). However, as we were not aware of this phenomenon while we were making our observations on *Inia* swimming together and our notes are not adequate to reconstruct the details of what occurred, the existence of echelon-formation swimming in *Inia,* though probable, cannot be considered as established.

BREATHING

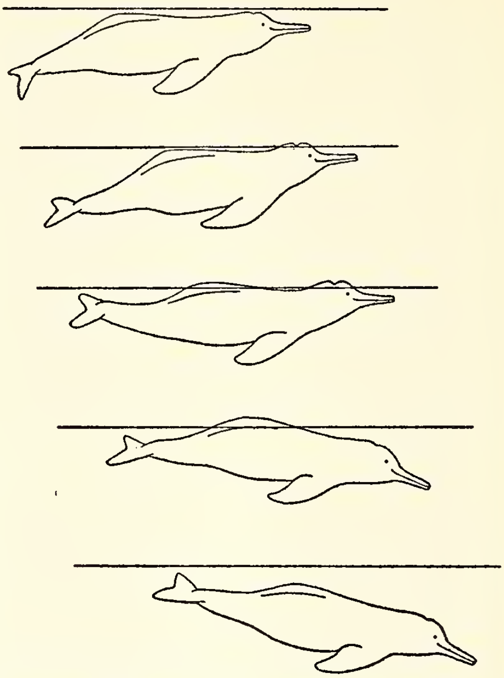
Under natural conditions *Inia* have been observed to surface for breathing in three ways (Layne, 1958). When swimming at the normal, slow cruising speed, the dolphins rise to breathe with the body parallel to the surface. The top of the head generally breaks the surface first and then the upper edge of the back lifts into view. The entire performance is accomplished in a leisurely and deliberate manner. Under some circumstances the dolphins roll in order in breathe, in typical marine dolphin fashion.

One type of roll, in which the animal arches high out of the water and re-enters almost vertically, appears to be associated with feeding activity, probably at some depth. The dolphins also roll to breathe when swimming rapidly. Under these conditions the body cleaves the surface in a low arc.

The captive *Inia* almost invariably utilized the horizontal method when surfacing to blow. Text-figure 1, drawn from a filmed surfacing sequence, illustrates this characteristic manner of respiration (Pl. II, Fig. 3). On several occasions when the dolphins had been alarmed and were swimming rapidly down the channel they performed somewhat abortive rolls, probably being prevented from executing the maneuver in the normal manner by the shallow depth of the water.

In typical surfacing, the head is brought out of the water only enough to expose the area about the blowhole; the beak does not ordinarily break the surface. The flexible neck permits the dolphin to lift the head to expose the blowhole when barely moving or even when motionless in the water. The closed blowhole is crescent-shaped, with the lateral points directed anteriorly. It has a more or less rectangular shape when fully opened. The opening of the blowhole appears to be brought about primarily by the forward movement and depression of the tissue mass associated with the anterior margin. In slow motion films of the action of the blowhole during the breathing cycle, we were unable to note the separate action of the plugs, vestibular sac and valve during the opening phase. When the blowhole was closing, however, the plugs, vestibular sac and valve could be seen operating in sequence. The size of the blowhole appeared to increase slightly, and the anterior margin to depress somewhat more, as inspiration progressed. Although our observations are not sufficiently detailed to permit critical comparisons, there appears to be no essential difference between the general functioning of the blowhole apparatus in *Inia* and that of *Tursiops* (Lawrence & Schevill, 1956).

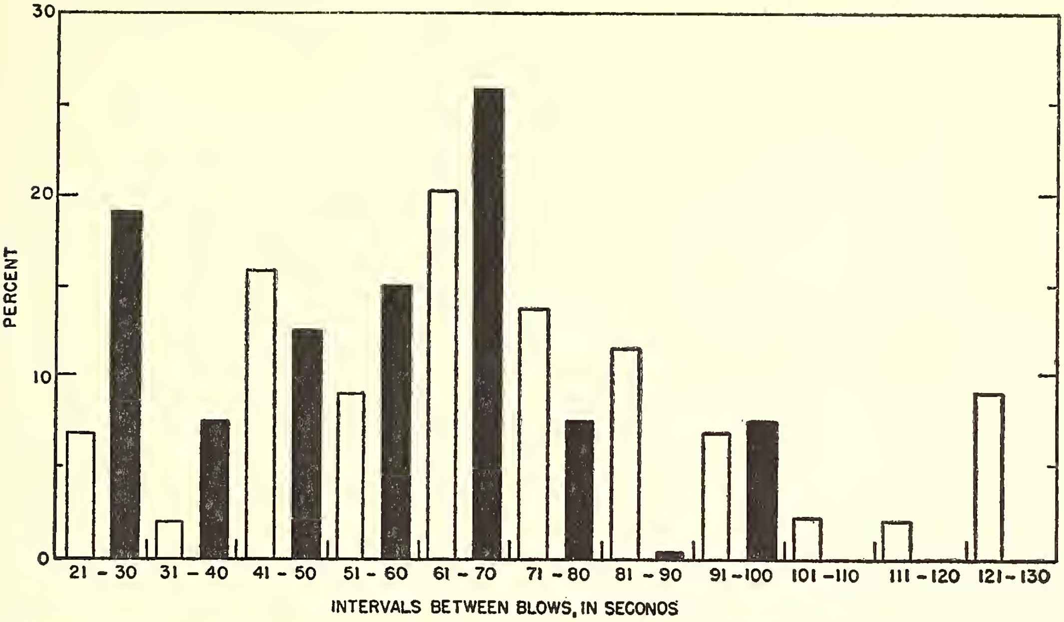
The blowhole of *Inia* remains open approximately 2 seconds (1.2 to 2.2 seconds in 23 instances timed with a stopwatch during a normal breathing cycle). Wild inias appear to spend about the same amount of time at the surface (Layne, 1958). Expiration and inspiration are of about equal duration and are typically accompanied by a sighing or gasping sound. However, the normal sounds of the expiratory and inspiratory phases are distinctly different. Occasionally air is released with an explosive snort instead of the usual fainter sound.



TEXT-FIG. 1. Typical breathing pattern of *Inia*.

The duration of the normal blow of *Inia* is apparently much prolonged in comparison with marine dolphins. Lawrence & Schevill (1956) recorded the times of eight blows of a single *Tursiops* loafing at the surface and found that the duration of the blow under these conditions ranged from only .47 to .78 seconds. Presumably, an active swimming individual of this species would blow even more rapidly. The melon of *Inia* is particularly conspicuous and produces a marked bulge in front of the blowhole which appears to deflect water away from the open blowhole when the dolphin is swimming at the surface (Pl. II, Fig. 4). This seems to represent an adaptation associated with the typical horizontal method of surfacing and prolonged breathing interval.

Intervals between blows of Amazon dolphins recorded in the field ranged from 5 to 112 seconds and averaged 32 seconds (Layne, 1958). We also made a number of observations on the frequency of breathing of the captive *Inia* under various conditions of activity. All intervals were timed with a stopwatch. Twelve records for the large male, while it was still on board the plane following its arrival from South America, ranged from 18 to 105 seconds, with a mean of 60. Four records for this individual as it rested on the bottom and rose at intervals to breathe ranged from 18 to 94 seconds, with an average of 58. Forty-four records for the large male while it was swimming normally averaged 71



Text-fig. 2. Frequency distribution of intervals between blows. Solid bars represent young male, open bars, the adult.

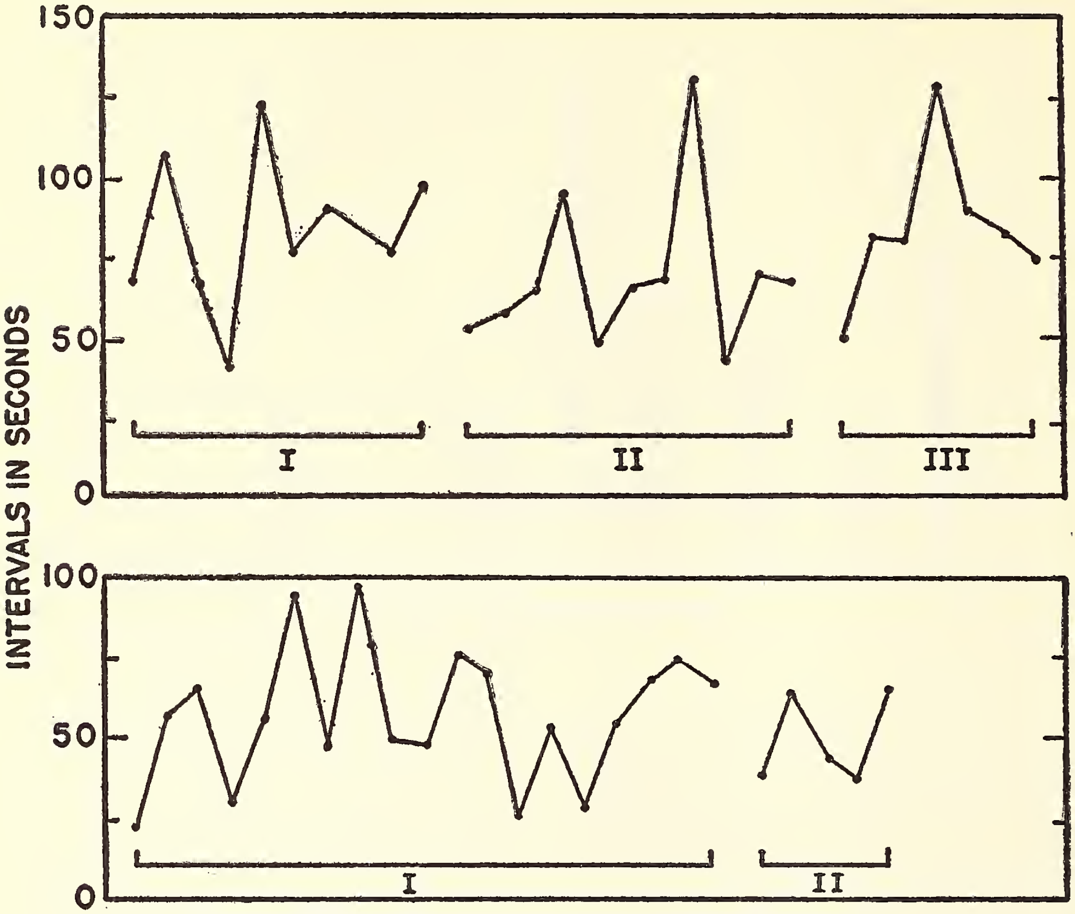
seconds (22-130) between blows. Twenty-six records for the small male under similar circumstances ranged from 22 to 98 seconds, with a mean of 54 seconds. The frequency distribution of the intervals between blows of the two captives during normal swimming activity is presented in Text-fig. 2. Although the data show no strong peaks at any particular frequencies, intervals between blows of greater than 80 seconds are relatively infrequent. These data also suggest that the small male had a higher breathing rate than the large one. Although approximately 32 percent. of the intervals of the latter were over 80 seconds, only about 8 percent. of those of the small dolphin exceeded this value. McBride & Kritzler (1951) note that infant *Tursiops* surface at nearly double the adult rate and attribute the difference to greater muscular exertion and heat loss in the young. A similar explanation may apply to the difference observed between the captive *Inia.*

In a number of instances we recorded the lengths of intervals between a series of consecutive blows. Several of these series, obtained under normal swimming conditions, are presented in Text-fig. 3. Although there is no clear-cut pattern to the periodicity of blowing, there does appear to be at least a slight tendency toward a sequence of several shorter intervals alternating with ones of longer duration. A similar pattern of breathing was suggested by observations on wild inias (Layne, 1958) and is clearly indicated in data for some delphinids (Norris & Prescott, 1961).

On several occasions we saw the large male release air when he was still a foot or more beneath the surface when coming up to blow. The air appeared to be released instantaneously in one large bubble, and sometimes the dolphin gave a loud blow when his head broke the surface. Once after the animals had been placed in the main spring, we observed the lips of the blowhole of the small male relax and gape slightly as the animal approached the surface to breathe but was still several feet underwater. However, no air was seen to escape in this instance. When disturbed while breathing at the surface, the dolphins would instantly close the blowhole and submerge without completing the cycle.

Infrequently, as a dolphin came to the surface to blow, a spout of water or cloud of fine spray would accompany expiration. The former appeared to be the result of beginning expiration before the blowhole cleared the surface, while the appearance of spray seemed to be associated with a particularly vigorous exhalation. The latter phenomenon might represent cases in which water present in the vestibule was being blown out, as described for *Tursiops* by Lawrence & Schevill (1956).

Often during the early period of their captivity, the two dolphins surfaced to breathe in nearly perfect synchrony (Pl. III, Fig. 5). We



Text-fig. 3. Intervals between blows in five breathing sequences.

also noted that when the animals were first placed in the enclosure in the main spring, they surfaced to breathe at the edge of the hyacinth mat or well out in open water. However, after a time they did not hesitate to push up through the mass of plants in order to expose the blowhole.

With the exception of Anderson's (1878) account for *Platanista,* details of the respiratory behavior of other platanistids are unknown. A captive *Platanista* observed by Anderson would rise slowly to the surface, exposing the blowhole and a portion of the back, blow and then slowly subside. Inspiration was so rapid that the blowhole seemed to close immediately after expiration. The entire breathing cycle took no more than a few seconds. A characteristic blowing sound, which is the basis of the various native names (*e.g.,* sus, susu, sihoo, etc.) applied to this form, accompanied breathing. The intervals between breaths ranged from about 30 to 45 seconds. According to Anderson, wild *Platanista* surface to blow in the same manner but may also plunge out of water upwards, forwards, and downwards, the snout being the first part of the body to appear. The usual surfacings are leisurely executed. On the basis of these observations, the mode of breathing of *Inia* and *Platanista* is apparently similar.

Hoy (1923) states that, according to the Chinese, *Lipotes* makes a "peculiar roaring sound," presumably when blowing, which is apparently of the same general nature as the sound produced by *Platanista* and *Inia.* This might indicate that *Lipotes* resembles *Platanista* and *Inia* in other aspects of breathing behavior as well.

Jardine (1837) states that *Inia* surfaces more frequently than marine dolphins, but does not give the evidence for this conclusion. McBride (1940) reports that *Tursiops* breathes about once every 30 seconds when sleeping and that the rate is slightly higher during normal activity. The mean values of respiratory intervals given for *Tursiops gilli* and *Orcinus orca* by Norris & Prescott (1961) do not differ greatly from those of *Inia.*

SENSORY CAPACITIES

Our information on sensory capacities is based upon observations of the behavior of the dolphins in various situations in which a particular type of stimulus seemed to be predominant. If the animal altered its behavior in a manner that seemed appropriate at the time the stimulus occurred, it was generally considered to be responding to the stimulus in question. For example, if a pebble was thrown into the water behind a cruising dolphin and the animal then suddenly speeded up its swimming, it was assumed that hearing and not vision was the sensory pathway involved. In actuality, many observations could not be given so obvious an interpretation. The increasing evidence of truly remarkable sensory phenomena in diverse kinds of animals, of which echolocation in marine odontocetes is an example, clearly illustrates the necessity of exercising great caution in drawing conclusions about the kinds, efficiency, and relative importance of the sensory modalities of an animal on the basis of general observations alone.

The principal sensory pathways utilized by the Amazon dolphin to obtain information about its environment appear to be vision, hearing and touch.

Vision.—A trend toward the reduction of the eyes, presumably correlated with life in turbid waters, is evidenced by the living representatives of the Platanistidae. This trend reaches its extreme development in *Platanista,* which is believed to be totally blind. A captive observed by Anderson (1878) would bump into the sides of its container when swimming about and showed no response when objects were passed rapidly before its eyes. Morphological evidence for absence of sight in this form is the lack of a crystalline lens and a rudimentary optic nerve. Numerous glands and structures resembling touch receptors of the skin occur in the conjunctiva, suggesting that the eye has become secondarily adapted to a tactile function (Anderson, 1878). Other than that it has small eyes (Burmeister, 1864-69), nothing is apparently known of the status of vision in *Pontoporia.* Allen (1938) states that the eye of *Lipotes* is very small and degenerate and that the dolphin is blind or nearly so. The latter conclusion appears to be based on the general appearance of the eye rather than upon actual observations of living individuals, although Hoy's (1923) brief account of the feeding habits of the white-flag dolphin suggests that sight may be of little importance.

Although the eyes of *Inia* are reduced as in other platanistids, they nevertheless appear to be completely functional. Gross reactions to light were noted on two occasions. When the large male was being photographed in the dimly-lighted interior of the plane after its arrival at Ocala, it jerked convulsively each time a flashbulb was discharged. After the dolphins had been in captivity two days, we visited them at night. Approaching the channel quietly in the dark, we waited until the dolphins came close then turned on our flashlights. As soon as the lights shone on them the animals panicked and swam rapidly away. Within a few minutes, however, they became accustomed to the lights and behaved in normal fashion.

Although the extent to which underwater vision serves *Inia* in the generally murky waters of its natural habitat is uncertain, the captive dolphins appeared to employ their eyes underwater to a considerable extent. When they were cruising about in the channel or pen in the main spring, they often seemed to be engaged in carefully scanning the botton as they passed over it. Frequently they would reach down almost perpendicular to the body axis, or actually back under the body, to seize a dead fish, stick, old paper cup, or other object lying on the bottom. The dolphins appeared to sight such objects, which were often partially buried or nearly hidden by vegetation, from a distance of at least 2 feet. On one occasion an *Inia* was seen to swim up to investigate a small leaf, not more than an inch in length, that was floating on the surface. Many of the activities involved in play appeared to be mediated by vision, and the animals seemed to watch the observer when being fed from the hand. While in the enclosure in the main spring, the small male was often attracted by persons swimming outside the fence, usually at least 50 feet away, and would swim to the fence as if to watch them. Reed Parham, a Silver Springs employee, told us that on one occasion he quietly swam underwater to the dolphin pen at a point along the fence where he was hidden from the animals inside by a thick screen of eelgrass. He then carefully thrust his hand through the vegetation and moved his fingers. At once the small dolphin began to swim directly toward him. When a few feet away, the animal appeared to close its eyes but continued to approach slowly, performing the characteristic head swinging movements. The interpretation of this observation is not clear. The general behavior of the dolphin suggested that he had seen the hand when it appeared. However, the fact that the animal appeared to close its eyes when coming close yet continued on a direct course offers the possibility that echolocation might also have been involved.

Although wild inias sometimes bring their heads far enough out of water during surfacing to expose the eyes and appear to examine their surroundings (Layne, 1958), such behavior was not observed in the captives. Nor did we find during the course of our study that the dolphins responded visually to objects above water when their eyes were submerged. One way in which this was tested was to wave a white handkerchief tied to the end of a stick a few inches above the water when a dolphin was approaching. In no case did either animal show any indication of being aware of the fluttering cloth just above its head, even as it passed beneath it. If the handkerchief was slowly and gently lowered into the water next to the bank while the dolphins were at the far end of the channel and held motionless except for movements caused by the current, the animals would seem to become aware of its presence from some distance away as they swam back down the channel. They would turn their heads in the direction of the handkerchief as they passed by at a distance of 5 or 6 feet. Townsend (1914) likewise found no evidence that *Tursiops* responded visually to objects above the surface when its eyes were under water, the dolphins he observed paying no attention to a fish suspended 2 inches above the surface.

The dolphins sometimes appeared to swim with the eyes closed during the day, although because of the small size of the eyes and their greyish coloration under water, it was difficult to be certain of this point. However, when the small male was being kept in the small tank before his death he sometimes surfaced to breathe with the eyes closed. This individual gave no response to a touch on the skin around the eyes nor on the eye itself. In contrast, Kritzler (1952) found that stranded pilot whales, *Globicephala,* were very sensitive to a touch on the skin around the eye and would immediately close the eye when thus stimulated.

In comparison with such marine dolphins as *Tursiops* and *Globicephala, Inia* does not appear to be a less visual animal to any significant degree. McBride & Hebb (1948) state that *Tursiops* uses its eyes both in and out of water, although it is not always easy to determine whether vision or audition is being utilized under water. Schevill & Lawrence (1956) noted that a captive individual studied by them used vision to locate fish at close range but apparently had difficulty in discrimination. Both *Tursiops* and *Globicephala* appear to have relatively keen vision above water and may employ the eyes in this way to a greater extent than *Inia.* This certainly appears to be true in the case of captives. Captive bottle-nose dolphins frequently thrust the head out of water or "pitch-pole" out in order to examine their surroundings, and the remarkable ability of trained individuals to catch and throw objects with great accuracy further attests to the acuity of above water vision. McBride (1940) credits individuals with apparently being able to see moving objects at a distance of 50 feet. Schevill & Lawrence (1956) noted that a captive *Tursiops* looked above water to inspect its surroundings with greater frequency during the early period of confinement than later, and according to Kritzler (1952) a captive pilot whale was also apparently able to associate what it saw above water with discrete situations. Observations of wild *Tursiops* herding mullet toward steep banks in an endeavor to trap them (Kritzler, 1952) and killer whales scrutinizing ice floes for seals (Norman & Fraser, 1949) indicate that marine cetaceans may use their eyes above water under natural conditions.

Hearing.—The impression gained from our experiences with the captive inias is that, as in the case of marine cetaceans, hearing is probably the most important sensory faculty possessed by these animals. Several observations suggested that the dolphins could hear above-water sounds, but were less sensitive, or acclimated more readily, to these than to sounds made in the water. The large male was seen to flinch violently at any loud sound when he was out of water and being transferred from the plane to the springs following his arrival from South America. Also, on the second day in captivity this individual reacted in a similar manner when a person standing on the bank some 15 feet away closed a camera case with a loud click. The dolphin was swimming below the surface at the time. On later occasions, however, neither dolphin exhibited any response when under water or at the surface for air to such sounds as the rapping of sticks together, clapping or the blowing of a small horn, although the same sounds if made under water would inevitably send the animals off to another part of the channel in panic.

The dolphins were extremely sensitive to under-water sounds; they invariably reacted to such sounds with a startle or flight response—the animal giving a convulsive twitch and immediately accelerating its swimming. A similar response to underwater sounds has been described for *Tursiops* and *Stenella* by Kellogg & Kohler (1952) and Kellogg (1953). The dolphin would always continue on the same course, at least initially, even though the sound came from in front. The dolphins reacted in this way to even the slightest disturbance in the water. A cigarette, a match stick, or small piece of paper falling on the water within 10 feet of an animal, slight movements of a small hollow pipe stuck into the bottom at the edge of the channel, and insects touching the surface of the water a few feet away were among the stimuli eliciting the startle response.

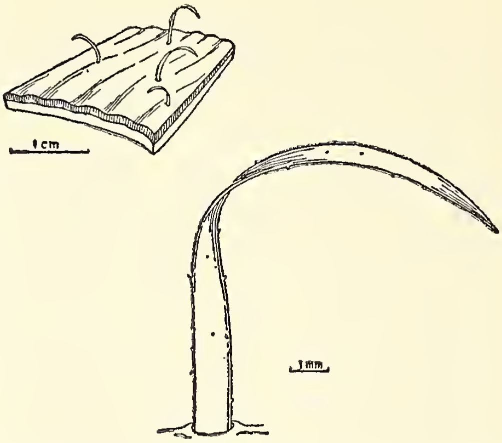
The inias were remarkably good at localizing the source of a sound. After a short time in captivity, they became conditioned to the splash of a fish being thrown into the water at feeding and would immediately swim rapidly to the source of the sound from any place in the channel. If at such times when the animals were excited and responsive, a stone was tossed into the water or the surface slapped with the hand or a stick, the dolphins would swim unerringly to the precise point from which the sound emanated, even though there were no ripples or other sign to mark the spot by the time they arrived in the vicinity. Natives in the Amazon region report that *Inia* can often be lured close to a boat by slapping the water with a paddle. Captive bottle-nose dolphins also respond readily to a slap on the water and localize the sound with great precision (McBride, 1940; Lawrence & Schevill, 1954; Schevill & Lawrence, 1956). Wild *Tursiops* may also locate prey in this way as well.

Field data and the observations made on the captives leave no doubt as to the extremely good auditory acuity of the Amazon dolphin and its refined ability to accomplish passive localization of a sound source. Although our evidence is admittedly circumstantial, we strongly suspect that *Inia* also possesses the ability to utilize active echolocation as one of its auditory orientation mechanisms. Several times during attempts to capture wild inias with a large net in a turbid channel or lakes near the Amazon, individuals seemed to perceive and avoid the net under conditions that seemed conclusively to rule out the possibility that vision was involved (Layne, 1958). The nature of the head movements often made by the captive dolphins when swimming about is also suggestive of the use of echolocation. When the dolphins were idling along the head was usually in motion. Movements in a horizontal plane predominated, the head being swung from side to side through a fairly broad arc, although it was also moved up and down to some extent. If the animal swam on its side, the nodding component of the head increased. The extent of head oscillations was keyed to the activity of the dolphins. When the animals were in an excited state, as during feeding or when disturbed, the head movements became more pronounced and rapid. An increase in the frequency of head-fanning was also observed as a dolphin approached a fish or other object that had been thrown into the water. In such instances the dolphins would often reduce their speed during the last few feet of their approach and move forward slowly while sweeping the head vigorously from side to side. If the target object was settling rapidly, the general direction of the head oscillations would sometimes seem to lag somewhat behind the object. An observation that may indicate similar behavior in wild *Inia* has previously been described by Layne (1958). In this case an *Inia* was seen to submerge, and a short time later a small fish jumped from the vicinity in which the dolphin had been. An instant later the open jaws of the *Inia* appeared, wavered back and forth a few times, then disappeared again. The general impression gained from these events was that the dolphin had been approaching the fish very slowly and engaging in the same head-wagging behavior as observed in the captives.

Head movements apparently similar to those described for *Inia* are associated with echoranging by the bottle-nose dolphin (Schevill & Lawrence, 1956; Kellogg, 1958, 1959). Kellogg (1960, 1961) has termed this behavior "auditory scanning" and states that it occurs only while a porpoise is emitting trains of sound pulses. The significance of this behavior apparently lies in the increased precision of binaural localization in determining the direction from which echoes are coming. The seemingly close similarity of both the head movements of *Inia* and the conditions under which they are exhibited to those of echoranging *Tursiops* provides perhaps the most convincing evidence for the use of echolocation by the former. The recordings made of *Inia* vocalizations possessed a great deal of background noise and were difficult to interpret. In our study of these recordings we were unable to identify sounds of the type associated with echolocation in *Tursiops*.

Touch.—Tactile sensations are evidently important to cetaceans. Captive specimens of *Tursiops* and *Globicephala* frequently engage in rubbing their bodies against other animals or on inanimate objects. In some cases this behavior may be the result of irritations of the skin, but in others the animals appear to derive sensual pleasure from the activity (Kritzler, 1952; Lilly, 1961.) As noted previously, *Inia* exhibits similar tendencies.

Reference has been made earlier to the manner in which the dolphins would often probe and stir the bottom with the beak as they swam along or use it to explore the crevices of the old bridge in the channel. This use of the beak suggests that it may have an important function as a tactile organ. This supposition is further strengthened by the presence of numerous stout hairs on the beak, a characteristic in which *Inia* differs from all other cetaceans. These hairs are flattened, relatively non-tapering, and possess a slightly spinulose surface (Text-fig. 4). They are usually erect at the base but may be bent over at the tip. The tip is sometimes uneven, as if from wear. Each hair is set in a small pit and appears to be rather freely movable. The morphological nature of these hairs strongly suggests that they are specialized for a tactile function and may thus increase the over-all efficiency of the beak for this purpose. Among the other genera of platanistids, both *Platanista* and *Lipotes* also utilize the beak for probing and stirring around in the mud. Of interest in this connection is the fact that the trigeminal nerve was well developed in zeuglodonts and some squalodonts and was probably associated with a good sense of touch in the muzzle area (Kellogg, 1928). As the squalodonts may be close to the ancestral stock of the Platanistidae, the presumably highly developed tactile sensitivity of the beak in the freshwater dolphins might be regarded as a primitive as well as an adaptively significant feature.



Text-fig. 4. General and detailed views of bristles on the snout of *Inia*.

Tameness and Learning Ability

During the early period of captivity, the dolphins would not allow any one in the water to approach them closely. They would become visibly agitated when the observer was still 50 or 60 feet away, and their minimum flight distance was approximately 10 feet. Moreover, they ordinarily swam off in alarm before the observer, even though moving slowly and cautiously, could get that close. When forced to swim past a person in the water, the dolphins would keep as far away as possible and roll onto the side and with the back directed toward the human. The two individuals often stayed close together at such times.

By the end of three months in captivity, the dolphins had grown quite tame. Both individuals would readily swim up to a person in the water at feeding time. The small male would take fish from the hand without hesitation. The larger, however, was less bold and would accept a fish only if the feeder released it and allowed it to float a few inches from the hand. The larger male also avoided being touched, while the small one permitted himself to be patted and stroked. He would also allow a human to climb upon his back and would follow a person around the pen at feeding time. On one occasion, he attempted to initiate play with an observer in the water with him.

The dolphins were quick to note the presence of new inanimate objects in their environment or changes in the relations of those already present. Their first reaction to a strange object was often one of fear and avoidance, and they frequently seemed to regard such an object with suspicion for some time after its appearance. Often when alarmed the pair would "close ranks" and remain together until the disturbance was removed or they became acclimated to it. McBride & Hebb (1948) have described similar behavior in *Tursiops* and point out that the degree to which such visually aroused fears are exhibited and their persistence show a relationship to phylogenetic development. The reaction of *Inia* to strange objects may not, however, be as pronounced as that of the bottle-nose dolphin.

One instance of a fear response to another animal was observed while the dolphins were in the channel. It was noted one day that the animals had begun to avoid the vicinity of the old bridge. Even when hungry, they were reluctant about coming to this area for food, and after seizing a fish would promptly swim to another part of the channel to eat it. A few days later a 4-foot alligator *(Alligator mississipiensis)* was discovered living in a hole beneath the bridge. The dolphins continued to avoid the area for some time after the reptile had been captured and removed. The behavior of the captives in this situation suggests the possibility that caimans may be one of the natural predators of *Inia.* McBride & Hebb (1948) describe behavior of *Tursiops* in the presence of tiger sharks *(Galeocerdo cuvieri)* that also suggests response to a natural enemy.

The *Inia* were initially induced to feed by throwing fish into the water near them. Within a few days they learned to associate a splash with feeding and would come from distant parts of the channel in response to a slap on the water with a hand or stick. The speed with which the animals learned to associate this sound signal with feeding cannot entirely be attributed to rapid learning ability, as they probably possess an innate tendency to utilize splashes in localizing prey. No effort was made to give the animals more complex training.

When in the enclosure in the main spring, the dolphins exhibited considerable curiosity about the activities of swimmers nearby. The young male was particularly inquisitive and would often swim along the fence trying to keep as close as possible to a swimmer on the outside. Wild *Inia* also appear to be inquisitive and may closely approach boats and appear to look above water to examine the occupants (Layne, 1958). However, if persecuted they may become shy and alert to signs of danger. Lamb (1954) observed the rapid disappearance of a school from around a boat when a rifle was brought on deck. Lamb also gives another account of the way in which wild *Inia* may associate themselves with human activities. In this case, a dolphin would appear on the fishing ground of a native fisherman, presumably in response to the rapping of a paddle on the side of the canoe and a peculiar whistling call. The animal would then accompany the boat, holding a station 50 to 100 feet away on the deep water side and apparently scaring fish back into shallow water or in some instances actually onto the beach. Alpers (1961) summarizes information on marine delphinids giving assistance to humans engaged in fishing or whaling activities.

Data on several species of smaller marine odontocetes indicate that, although species differences do exist, these animals are in general easily tamed and learn rapidly (Brown & Norris, 1956; Brown, 1960; Hediger, 1952; Kritzler, 1952; Lawrence & Schevill, 1954; McBride, 1940; McBride & Hebb, 1948; McBride & Kritzler, 1951). There is, however, little information available on the actual time taken for wild-caught individuals of these species to become tame and to learn particular tasks, although Brown (1960), Kritzler (1952), McBride & Kritzler (1951), and Lawrence & Schevill (1954) give some data on these aspects for *Tursiops* and *Globicephala*. On the basis of the present observations, *Inia* does not appear to differ in any significant degree from the marine forms in the rapidity and extent to which it becomes tame nor in the general development of its learning ability, at least with respect to such simple things as taking food from the hand and responding to a sound as a signal for feeding. Although no effort was made to give the Amazon dolphins the more complex type of training that some of the marine odontocetes receive in the various oceanariums in this country, we believe that *Inia* would show comparable learning capacities in those tasks that did not depend upon speed and agility, in which respects it is much inferior to forms like *Tursiops*.

FEEDING BEHAVIOR

Although the Amazon dolphin has been considered to be entirely piscivorous (Jardine, 1837; Kellogg, 1940), there are few data on its food habits in the wild. The stomach of a specimen from the Rio Guapore in Brazil examined by Natterer contained the remains of fish (Pelzeln, 1883), and *Inia* have been observed in apparent pursuit of fish (Layne, 1958). Additional information on foods was provided by the stomach contents of the small female that died en route to Florida from Colombia. This animal was captured on March 5, held overnight in a small fish pond, and loaded on the plane for transport to the United States the following day. Since there was no evidence that she had fed while in the pool, the stomach contents were presumed to represent feeding prior to capture.

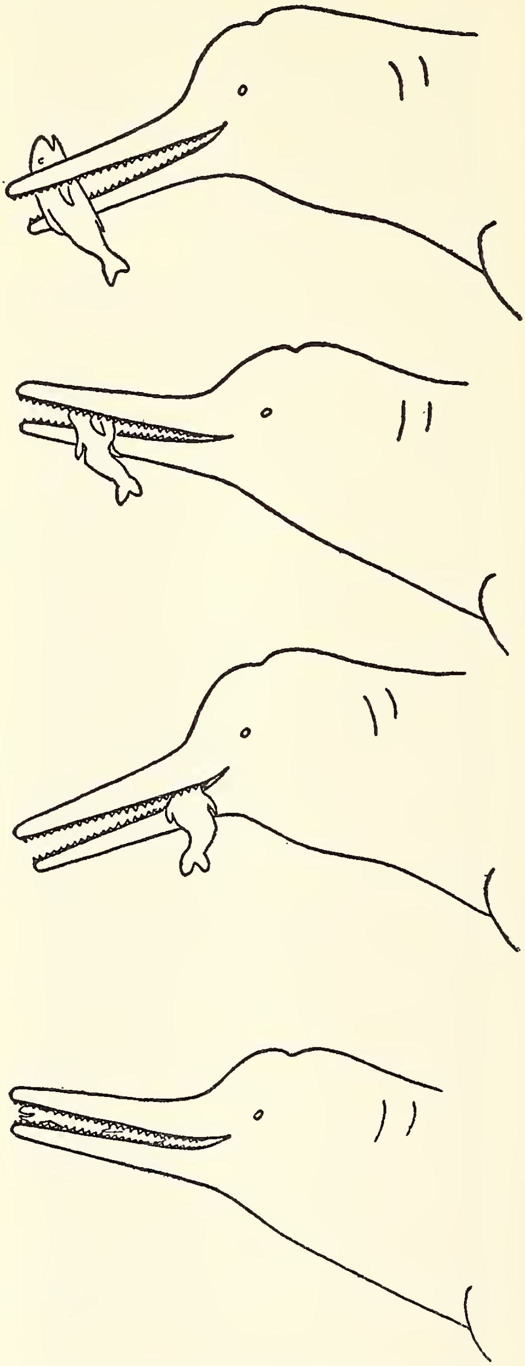
The stomach of this specimen contained about 1½ quarts of fish remains. The bodies of several of the fish were nearly intact, though badly crushed, whereas the rest were fragmentary. The fish were judged to have ranged in size from about 4 to 8 inches and were either all of the same species or of closely similar types. Several of the more complete specimens were tentatively identified as being probably of the genus *Myleus*, one of the serrasalmine characins closely related to the piranhas. In addition to the fish remains, the stomach contained some insect fragments, chiefly beetle elytra and wings, a large seed, and a small amount of vegetation. The latter consisted of rather compact balls of what appeared to be the linear leaves of an aquatic plant. The insect and plant material comprised only a very small amount of the material in the stomach and in all probability resulted from accidental ingestion.

The captive *Inia* were initially fed on live minnows and sunfishes about 5 or 6 inches in length. The fish were thrown into the water near the dolphins with enough force to stun them and thus make them easy prey for the cetaceans. The large male fed on fish offered in this way the day following his arrival at Silver Springs. By the next day he had learned to associate the smack of the fish on the water with feeding and would come readily from some distance away upon hearing the sound. Although he would respond to the splash from a distance of 25 feet, he did not appear actually to detect the fish until within 6 to 10 feet. If a fish recovered and swam off before the dolphin arrived, he would often circle the area in search of it.

As soon as the dolphins were feeding well on small fishes, their diet was changed to dead blue runners (Carangidae) and mullet *(Mugil)* which were obtained fresh daily from a local market. Together, the dolphins consumed between 20 and 35 pounds of fish daily. At first they were fed at frequent intervals throughout the day, but later their feeding schedule was reduced to a single feeding a day. When on a multiple feeding schedule, the dolphins usually showed greatest interest in food at the first feeding in the morning, but they would continue to accept food during the remainder of the day. The pair would usually consume about 5 pounds of fish at a feeding. When hungry, the dolphins were extremely responsive to a splash made when a fish was thrown into the water and would swim rapidly to the spot from any part of the channel. Sometimes they would become quite excited during the feeding period and would blow more frequently and hurriedly than normally. They often seized the fish at the surface before it began to sink or as it floated toward the bottom. They would also regularly pick up fish from the bottom, sometimes only to play with them but at other times to consume them. In many instances the fish had laid on the bottom for some time and had become nearly covered with silt.

The dolphins showed a preference for fish under a foot in length. The manner in which a fish was consumed was quite characteristic (Text-fig. 5). Almost invariably the dolphins would seize their prey with the tip of the forceps-like jaws (Pl. III, Fig. 6). The fish was then allowed to slip to the rear of the jaws where it was moved from side to side and vigorously chewed (Pl. III, Fig. 7). It was then manipulated into a longitudinal position in the jaws and swallowed headforemost. Occasionally, a dolphin would make a sharp turn to aid in swinging the fish into the proper position for swallowing. Immediately after swallowing a fish, the dolphins would often make short, quick nodding movements with the head. The captives always swallowed their food underwater, although wild *Inia* have been observed apparently consuming their prey at the surface (Jardine, 1837; Layne, 1958).

When swimming rapidly, the dolphins would occasionally seize a fish at the rear of the jaws rather than the tip. Sometimes an individual would carry a fish in the jaws for a minute or two, even surfacing to blow, before swallowing it. While carrying a fish in this manner, the dolphins would frequently toss it about in the jaws and occasionally even release it and allow it to drift some distance away before retrieving it with a slow, deliberate movement. When passing the fish to the rear of the jaws for masti-



TEXT-FIG. 5. Sequence showing typical method of grasping, chewing, and swallowing a fish utilized by *Inia.*

cation the dolphins often allowed it to drift passively back into position rather than manipulating it with the jaws. The rather careless manner in which dead fish were usually handled by the captives after the initial bite suggests that perhaps in natural feeding *Inia* ordinarily cripples or kills its victim with the first bite so that there is little danger of it escaping while being maneuvered into position for crushing and swallowing.

While they were in the channel, the *Inia* always swallowed the fish entire after thoroughly chewing it. After they had been transferred to the pen, however, they showed a tendency to break up the fish into several pieces before swallowing. Sometimes the head was discarded.

We observed only one instance of natural feeding. This occurred while we were watching the large male with flashlights on the third night after he had been put into the channel. The animal broke the surface several times and once swam rapidly up to the bank as if in pursuit of something. A short time later it was seen carrying a fish about a foot in length. The fish appeared to be either a gar *(Lepisosteus)* or pickerel *(Esox)*. The dolphin swam from below the bridge to the upper end of the channel with the fish held crosswise in its jaws but when it came back down the channel past the point from which we were watching the fish was gone. We could not determine whether the dolphin had dropped or swallowed it. A few minutes later the dolphin caught a gar about a foot long. The manner of capture was clearly observed. The fish was suspended motionless in the water when the dolphin approached. The latter did not increase its speed or show any other indication of being aware of the fish's presence until nearly abreast of it and about 2 feet away. It then swung its beak around in a deliberate motion and neatly seized the fish with the anterior part of the jaws about 2 inches back from the tip. The fish made no effort to escape until actually gripped in the dolphin's jaws. The *Inia* held the gar crosswise in the mouth and proceeded to shake it vigorously. It then released the fish which, although badly crippled, swam feebly away. A short time later the *Inia* captured another gar of about the same size as the previous one, possibly the same individual. Again the dolphin seized the fish with the front of the jaws and shook it terrier fashion. This time, however, it passed the fish to the rear of the jaws, chewed it vigorously, flipped it lengthwise in the mouth, and swallowed it headfirst.

Although no further instances of natural feeding were observed, an *Inia* would occasionally be seen to "track" a small fish swimming across its path but without making any effort to capture it. From time to time dead fish, including sunfish (Centrarchidae), eel (*Anguilla*), catfish (Ictaluridae), and gar and water snakes *(Natrix)* were found in the dolphins' pen in the main spring. The animals had presumably been killed by the *Inia,* but there was no sign that the dolphins had made any effort to consume them.

Almost nothing is known of the feeding behavior of *Inia* under natural conditions. Field observations have indicated that the dolphins may forage either at some depth or near the surface and that the prey may be caught either by direct chase or slow stalking (Layne, 1958). The type of fishes found in the stomach of the small female also supports the contention that the dolphins may feed in the pelagic zone. The captives showed no hesitation in taking dead fish at the surface or while floating toward the bottom, and the observed captures of live fish occurred at intermediate depths.

As noted above, however, the captives frequently swam along near the bottom and would probe and dig in it with the beak. They would frequently pick up an object and toy with it and often ate dead fish off the bottom. It is possible that *Inia* forages in a similar manner in its natural environment. In fact, its small eyes and the generally turbid conditions of the waters it inhabits suggest that it may feed extensively in this manner, using the long beak to locate and dislodge bottom-dwelling fishes. The flexibility of the neck probably permits the beak to be used more effectively, and its vestiture of hairs probably increases its tactile sensitivity.

Wild *Inia* apparently capture and manipulate their prey in the same way as do captives. Circumstantial evidence for this was provided by the old female obtained from Tarpon Zoo. In this specimen, the teeth in the part of the jaws usually employed by the captives in seizing their prey were broken and worn, in distinct contrast to those in the remainder of the tooth rows (Plate IV, Fig. 8).

The nature of the dentition of *Inia* is clearly correlated with its mode of feeding. The anterior teeth are simple, conical structures; whereas the posterior series in each jaw are broad and heavy and have a rugose surface and a prominent internal projection. The form of the posterior teeth of *Inia* is unique among living cetaceans. The teeth in the anterior region of the jaws, where the prey is typically first seized, are well suited to a grasping and holding function, while the molariform rear teeth are admirably adapted for mastication. The characteristics of these teeth suggest that *Inia* is specialized for feeding upon hard-bodied prey. When further information on the natural foods of this dolphin becomes available, it may be found to feed extensively on armored or heavily-scaled fishes, such as certain of the South American catfishes, or perhaps even on mollusks or crustaceans of some kind. Our observation of a captive eating a gar shows that the dolphins are capable of taking hard-scaled prey.

In connection with their fluviatile habitus, there has been a general trend toward the development of bottom-feeding habits accompanied by reduced vision and greater reliance on the sense of touch among the living plantanistids. Apparently the least specialized in this respect is *Pontoporia* which is the only form ranging into estuarine and coastal waters. The teeth of the La Plata dolphin are numerous, conical and slender, apparently specialized for grasping and holding relatively weak, soft-bodied prey. Its diet is reported to consist of cephalopods and various kinds of fish, including mullet and croaker-like species (Kellogg, 1940; Cabrera & Yepes, 1940). *Lipotes* apparently feeds largely on fishes that it stirs out of the bottom muds with its elongate snout. Hoy (1923) obtained about two quarts of a species of eel-like, mud-dwelling catfish from the stomach of a specimen he collected. *Platanista* is the most highly specialized for bottom feeding of any of the platanistids. It apparently feeds almost exclusively by groveling in the bottom with the long snout. Since the eyes are degenerate, the Ganges dolphin probably locates its prey principally by touch (Anderson, 1878). The foods recorded for *Platanista* consist of several species of fish, including mud-frequenting species, and the crustacean *Palaemon carcinus,* which is also a benthic organism. In addition, such items as rice, seeds of other grasses, beetle remains, and an undigested bee have also been found in stomachs (Anderson, 1878). Such materials in all probability represent secondary or accidental ingestion. The present fragmentary data on the foods and feeding behavior of living platanistids, suggest that the feeding habits of *Inia* correspond most closely to those of *Lipotes,* with which it also shows closest taxonomic affinity. *Inia,* however, may be somewhat more pelagic in its feeding habits than *Lipotes.*

Sexual Behavior

The sexual behavior of the captive *Inia* appears to be essentially similar in kind and extent to that reported for captive delphinids (McBride & Hebb, 1948; Brown & Norris, 1956; Kritzler, 1952). The first instance of sexual activity was recorded after the dolphins had been in captivity about two weeks and involved masturbation by the small male. The incident was observed by Warren Prince and Paul Cunningham. The dolphin had been swimming around in circles over a patch of white sand when an erection was noted. The penis in the fully turgid condition was dark in color, pencil-shaped and slightly curved anteriorly. Its length was estimated as between 10 and 12 inches. About the time the erection was noted the animal rolled over and began to swim on its back. It then proceeded to manipulate the penis with both flippers, at the same time bending the head forward to observe its actions. Ejaculation was not observed.

Numerous additional instances of sexual activity were observed after this date, and the frequency of this behavior increased during the late summer. Both dolphins were often seen swimming with erections, and occasionally one would attempt intromission with the other. On one occasion, the large male was observed to settle onto a large tin can sitting on the bottom and then perform thrusting movements with its body. This was apparently another form of masturbation.

The frequency of sexual behavior declined markedly following the separation of the dolphins in the pen in the main spring.

Play

The *Inia* exhibited a variety of behavior that seems best considered under the category of "play." Play activity originated spontaneously, since the dolphins received no special training or encouragement in the development of play habits during the tenure of their captivity. Although some behavior that might be construed as play was observed within a few days after the animals had been brought to Silver Springs, the frequency and complexity of play behavior increased after they had been in captivity for several months. The small male was somewhat more active in its play and played more frequently than the large one. McBride & Hebb (1948) noted that partly grown *Tursiops* are more playful than adults.

After only two days in captivity the large male was seen to seize a small stick thrown into the water nearby and to take it below the surface before releasing it. Whether this incident should be regarded as actual play or merely as an interest in possible food is not known.

What seemed clearly to be play was first noted on March 31, when we saw the small male pick up a piece of fish, toss it about in his jaws, and then release it. Following this he picked up a piece of water-logged wood off the bottom and repeated the performance. As the wood slowly sank to the bottom after being released, the dolphin swam past it and deliberately rubbed it with his neck. Similar "toying" behavior in which the animals picked up dead fish or other objects off the bottom was subsequently observed on many occasions.

The dolphins also played with objects found at the surface. When in the pen in the main spring, they would often swim up and pull down a clump of hyacinths. After swimming around near the bottom, either upright or on their backs, and chewing the plants at the rear of the jaws, the dolphins would sometimes release the hyacinths, allow them to float part way to the surface then catch them again with a deliberate movement of the beak. Once a dolphin was seen swimming on its back near the bottom with a clump of hyacinths clasped in the axil of the flipper.

Sticks were a favorite play object. The dolphins would often play with a stick so vigorously and persistently that it would frequently be worn down and broken up into a number of smaller pieces. The small pieces of water-logged sticks found in the stomach of the small male at autopsy had apparently been swallowed during such play. When sticks or other buoyant objects were released under water, the dolphins would often swim after the object as it rose toward the surface and catch it with the forward edge of the flukes, keeping it delicately balanced there as they swam about.

After the dolphins had been placed in the main spring, the small male was observed playing with sticks above water. He would swim to the surface with a stick held in his jaws, thrust the forepart of his body out of water and toss the stick 4 or 5 feet away with a sluggish swing of the head. He would then quickly swim to the place where the stick had landed and retrieve it. This performance was repeated several times in succession. He also carried the stick around between the flipper and the body and would occasionally stand vertically in the water and toy with a stick lying on the bottom.

On one occasion an inflated rubber beach ball was thrown into the pen. The dolphins showed great interest in it and would often swim up beneath it and poke at it with the beak. After the ball was broken the large male was seen playing with a piece of it under water. He would swim about tossing it around and catching it again with the jaws, and he later tucked it into the axil of the flipper and carried it for some time in that manner.

In addition to individual play, the dolphins often engaged in joint play activity. The type of joint play most commonly observed consisted of passing a stick back and forth. This was first seen one evening after the dolphins had been in captivity about a month. One would seize the stick in its mouth and swim off with it. As he swam along he would let the stick go, and catch it between the flipper and body. He would then release it again and catch it on the edge of the flukes. When the stick would slip off the flukes the other individual would grab it and repeat the performance. Even after the dolphins were separated in the pen in the main spring, they continued such play by passing the stick back and forth through the meshes of the fence.

Several times the young dolphin was observed in an apparent attempt to entice the other to play. On one such occasion he would swim up behind the large male, touch the rear part of its body with his beak, and then swim rapidly away as if expecting pursuit. He would also approach the large male from the rear and swim rapidly and warily past him without making contact with the beak. After passing, the small male would often swim downstream a short distance and then swing into a cove where, partially hidden by vegetation, he would watch the large individual. When the latter came close, the small male would leave his hiding place and swim rapidly away.

The small male became tame enough to attempt to initiate play with human beings. This he would do by swimming up to a person in the water, clasp a hand tightly between the flipper and the body, and then attempt to tow the person along. This behavior did not appear until the animal had been in captivity about five months.

As noted in connection with feeding behavior, one of the captives was observed catching live fish, shaking, and releasing them, and on occasion fish or snakes that had apparently been killed by the dolphins were discovered in the enclosure. It is possible that such behavior might have represented play. Wyman (1863) and McBride & Hebb (1948) remark that both *Delphinapterus* and *Tursiops* often play with small fish in captivity, frequently catching them in the mouth and releasing them, and that sometimes the fish are injured in the process.

Lamb (1954) reports that wild *Inia* are said occasionally to come up beneath the boat of a lone fisherman and seize the paddle. Such behavior may fall within the category of play.

Observations on captive marine delphinoids of several genera, including *Tursiops, Delphinapterus, Globicephala, Lagenorhynchus* and *Delphinus* (Brown, 1960; Brown & Norris, 1956; Kritzler, 1949, 1952; McBride, 1940; McBride & Hebb, 1948; McBride & Kritzler, 1951; Townsend, 1914; Wyman, 1863), have revealed highly developed play behavior in both the young and adults. Although species differences exist, the play patterns that have been described are similar. The captive *Inia* exhibited most of the kinds of play reported for marine odontocetes, and play appears to be an equally important activity for them. Such differences as are apparent seem to have a structural rather than a motivational basis. For example, *Inia* does not appear to indulge in throwing objects above water as much as *Tursiops,* nor is it able to attain such distance and accuracy as the latter when it does attempt this type of play. This difference is apparently due to the fact that *Inia* is a much slower swimmer and more awkward than *Tursiops.* Brown & Norris (1956) have noted such differences, which also appear to have a structural basis, in the play behavior of *Lagenorhynchus* and *Tursiops.*

VOCALIZATION

Charles Hooper, one of the persons accompanying the first flight of *Inia* to Florida, reported hearing the large male utter squealing notes during the flight.

Two attempts were made to record sounds of *Inia* with a hydrophone during the early period of their captivity. Unfortunately, the dolphins engaged in little vocal activity on these occasions, and only two sounds are discernible on the recordings obtained (Schevill & Watkins, 1962). These include a loud rasping sound resembling a "bronx cheer" and a faint, plaintive yelping sound with a fundamental frequency of about 500 cps. The first sound may be comparable to the class of phonations termed "rasping and grating sounds" by Wood (1953) and reported by him for captive *Tursiops* and by Kritzler (1952) for *Globicephala.* Similar sounds are given by several of the delphinids included in the recordings of cetacean voices presented by Schevill & Watkins (1962). The yelping note of *Inia* is much lower in pitch than the majority of the odontocete vocalizations contained in the Schevill & Watkins' recordings. It most closely resembles certain calls of the white whale, *Delphinapterus,* the frequencies of which extend down to about 700 cps.

After the dolphins had been in captivity for some months, two additional sounds were heard by observers swimming underwater near the animals. These were described by Ricou Browning and William B. Ray as a high-pitched squeal and a loud "pow, pow, pow" made by snapping the jaws together. The sounds were heard only on infrequent occasions, and the circumstances under which they were emitted were not recorded in sufficient detail to permit drawing any conclusions as to their functional significance. Squealing vocalizations appear to be characteristic of many marine odontocetes, and the sound produced by closure of the jaws is clearly the same as the "jaw clap" recorded for *Tursiops* by numerous authors (McBride, 1940; McBride & Hebb, 1948; Tavolga & Essapian, 1957; Wood, 1953) and interpreted as a form of intimidation display.

As previously noted, the captives sometimes gave an explosive snort when at the surface for air. In some cases this seemed to be simply a sneeze but in others the sound was emitted under circumstances suggesting that it might be an expression of alarm, surprise, or anxiety and thus have a communicatory function. One observation that lends itself to such an interpretation involved the large male. He was visited on the second night in captivity, and shortly after the observers had taken a position on the bank of the channel the dolphin swam toward them and snorted three times in quick succession. On another occasion, when one of the *Inia* was at the surface for air, it gave the snort when a pipe stuck in the bottom nearby was moved. Lawrence & Schevill (1954) note that an old female *Tursiops* studied by them frequently gave explosive blows before she became tame. They suggest that the sound may have about the same meaning as the snort of a horse.

Little information is available concerning the sounds of other platanistids. The sighing sounds described for the Ganges dolphin are apparently the normal accompaniment of respiration. Hoy (1923) notes that a wounded *Lipotes* gave a " . . . subdued bellow, somewhat after the nature of the noise made by a buffalo calf." He also mentions that a roaring noise heard at night on Tung Ting Lake is also attributed to the white-flag dolphin by the Chinese. This might also be a respiratory sound.

RELATIONSHIPS BETWEEN THE PAIR

During the early period of their captivity, the two *Inia* usually remained close together. The small male almost invariably took the initiative in maintaining the association. In some cases the two individuals swam side by side, with parts of the body in contact. The possible relationship of this behavior to echelon-formation swimming has been discussed under Locomotion. Often the small male would trail more to the rear of the other and not in such intimate association (Pl. IV, Fig. 9). He would frequently caress the large male with his beak or flipper or brush against him with other parts of his body and sometimes cross from one side to the other over the caudal peduncle of his companion. If he fell behind, he would increase his pace in order to catch up and resume his previous position. Rarely did the large male attempt to regain contact with the smaller when the two became separated. In turning, the large male would nudge the small one with the head and beak if the latter was abreast of him (Pl. IV, Fig. 10). If the small male was in his typical position slightly to the rear, he would start his turn as soon as the large one began his without waiting for actual contact.

When the large male stopped to rest on the bottom, the small individual would follow suit. The latter was usually restless, however, and would move back and forth or nudge the large male with his snout, flippers, or other parts of the body.

During the early period of their captivity behavior suggesting antagonism between the dolphins was observed on only one occasion. The incident occurred while the large male was exploring the fence at the upstream end of the channel. During the time the large male was digging at the base of the fence and thrusting his beak gently through the wires, the small individual persisted in bumping him and nibbling at his flukes as if attempting to initiate play. Several times the big male, apparently annoyed, turned and gave chase to the smaller individual. Each charge carried the dolphins about 20 feet downstream. Once the small male lay on his side and arched his body as if in anticipation of an attack when the large male rushed at him, but in no instance was actual body contact observed. Prior to each charge, the large male released several large bubbles from the blowhole, and at one time during the period of observation a stream of small bubbles was seen escaping from the blowhole of the smaller dolphin. It is possible that the release of air noted in these instances was associated with the production of whistling or squealing vocalizations, as reported in several marine dolphins (Kritzler, 1952).

Usually the dolphins did not interfere with each others' feeding, even at times when they were swimming in close association. Often one would seize a fish off the bottom, toss it about a bit, then release it, whereupon the fish would be grabbed by the other dolphin and swallowed. In only two instances was a struggle over a fish seen. In both cases the dolphins were swimming together and the large male reached over and seized a fish being carried by the smaller individual. The small male attempted to hang onto the fish, the dolphins pulling and twisting it between them. In one instance the adult wrested the entire fish from the small male, and in the other the fish broke in two and each animal swallowed the piece it had retained.

After about a month in captivity, the pair began to exhibit a somewhat lessened tendency to associate together. Although the small male still trailed the other for considerable periods, he now began to make more frequent independent excursions to other parts of the channel. From this time on agonistic behavior between the pair increased. By the fall of 1956 it became necessary to separate the animals in the enclosure in the main spring, as the large male was persistently harrassing the other by chasing and biting it. A frequent maneuver of the large male was to swim up behind the other dolphin, seize his flukes, and pull him downwards and backwards. Such attacks were frequently carried out just as the small male was rising to the surface to blow.

No change in the behavior of the large male was seen after the younger animal died, except, as noted earlier, that it did not cross into the other side of the enclosure for several days after the fence had been lowered.

Cooperative behavior has been described for a number of cetaceans in the wild or under captive conditions (Hubbs, 1953; Siebenaler & Caldwell, 1956; Brown & Norris, 1956; Norris & Prescott, 1961). The degree to which this type of behavior is developed in *Inia* is unknown. Lamb (1954) recounts an instance of a herd of *Inia* gathering around one of their members that had been shot at, and Layne (1958) observed what appeared to be a somewhat similar case. The reaction of the large dolphin to a dead individual was tested on the first day of its captivity. While the dolphin was exploring the upper reaches of the channel, the body of the small male that had just died was slipped into the stream. Several minutes later the large male discovered the carcass on the bottom and swam slowly towards it until about 10 feet away. He then stopped and appeared to examine the body carefully, finally turning and swimming away. He repeated this performance twice more, once approaching quite close to the dead animal and appearing actually to touch its body with his beak. He made no attempt to "assist" the dead specimen in any way. Field observations indicate that *Inia* are not highly social and do not ordinarily associate in the closely-knit groupings of marine dolphins such as *Tursiops* and *Globicephala* (Layne, 1958). This apparent lack of a relatively strong social organization in *Inia* may thus be associated with a lesser tendency toward cooperative behavior.

DISCUSSION

The structural primitiveness of the platanistids raises the question of the status of their behavioral capacities as compared to those of more advanced odontocetes such as the delphinids. Although the bottle-nose dolphin, *Tursiops truncatus,* has been the subject of many studies and more is known about its behavior than about any other cetacean, information on the behavior of the numerous other members of the Delphinidae is scanty. The same is true of the Platanistidae, *Inia* being the only form for which even the broad outlines of behavior are presently known. Thus, while it is of interest to attempt to compare the behavioral development of these two groups in view of the differences in their apparent phylogenetic position, the limitations of the data are such that any conclusions reached must for the present be regarded as tentative.

Among the more conspicuous behavioral characteristics of *Tursiops* are an apparently high order of learning ability, great sensitivity and responsiveness to the environment, elaborate and persistent play activity in both young and old animals, a high level and great diversity of sexual behavior and well developed group-oriented behavior. Included in the last category are a tendency to associate together in schools, the establishment of individual "friendships" and several kinds of cooperative behavior involving food getting, defense against enemies and assistance to injured individuals of the same or other species. The general pattern of behavior typified by *Tursiops* seems to apply also to other delphinids that have been studied, although species differences clearly exist. In fact, the same trends, most of which have a social basis, may underlie to a varying degree the behavior of all cetaceans, reaching their greatest development in the smaller marine odontocetes and in the bottle-nose dolphin in particular.

In this paper we have attempted where possible to compare our observations on various aspects of the behavior of *Inia* with data available for *Tursiops* and the other small toothed whales that have been kept in captivity. In so doing we have been more impressed by the similarities, both general and particular, in the behavioral patterns of *Inia* and the other forms than by the differences.

Although the data are admittedly inadequate, *Inia* does not appear to be innately less capable of being tamed and learning certain simple tasks than delphinids and the white whale. Its ability to master more difficult tasks remains to be demonstrated, but it seems likely that its capabilities will be found to lie within the range of the marine forms. No evidence of possible "insightful" behavior comparable to that recorded for *Tursiops* (Caldwell, 1956; Brown & Norris, 1956) has yet been recorded in *Inia,* but this may reflect the difference in the degree to which the two forms have been studied rather than the fact that *Inia* is incapable of such behavior.

As is true of delphinids, the Amazon dolphin is alert and quick to perceive and respond to changes in its surroundings. The tendency of the captives to show fear of strange inanimate objects and to avoid them for a period of time resembles the behavior of the bottle-nose, common, and striped dolphins (McBride & Hebb, 1948; Brown, 1960). This trait, however, is apparently not consistent within the Delphinidae, as Brown (1960) did not observe it in a captive pilot whale.

The captive *Inia* also displayed the same type of play and sexual behavior described for marine odontocetes, and they seemed to be equally motivated to indulge in such activities. Because of its structure, *Inia* is less adept at certain kinds of play than *Tursiops.* Structural differences also seem to be responsible for variations in play behavior between species within the Delphinidae (Brown & Norris, 1956).

Several of the group-oriented behavioral characteristics of the delphinids also exist in *Inia.* Wild Amazon dolphins tend to travel in schools and show some development of cooperative behavior. In the captives, the occurrence of joint play and sexual activity, their close association during the early period of captivity, and the possible occurrence of echelon-formation swimming behavior may also be offered as examples of social development. The data available for *Inia* and delphinids also appear to indicate some basic similarities in vocal and other sounds that may play a role in communication. Jaw clapping, snorting, squealing, rasping calls and fluke-slapping may be cited as specific examples. It is also possible that further studies of *Inia* may reveal additional similarities in sound patterns to those of delphinids.

On the other hand, there is evidence that suggests that the social behavior of *Inia* is less well developed than that of *Tursiops* and perhaps the Delphinidae generally. Its schooling tendencies seem to be weaker, and one gains the impression of a lower level of interaction, such as cooperative behavior, between individual *Inia* in the wild than in many delphinids. The lack of evidence for the development of a close "friendship" between the captives as has been reported for *Tursiops* may be a further indication of a lower level of sociality in *Inia.* Our observations also suggest that the Amazon dolphin may be less vocal, in the sense of both the amount and variety of phonations, than *Tursiops,* and this might also be interpreted as evidence of weaker social orientation. Such a conclusion, however, must be considered highly tentative. As shown by the studies of Lilly & Miller (1961) on *Tursiops*, the conditions under which observations are made may have a decided influence on vocal behavior. Thus, perhaps under other conditions of captivity or with more animals involved, greater vocal activity might be shown by *Inia*. It is also possible that differences in vocal behavior among the Delphinidae may be as great as those which presently appear to exist between *Inia* and *Tursiops*. Wood (1953) found pronounced differences in the extent and variety of vocalization of captive *Tursiops* and *Stenella* which he suggested were correlated with ecological factors in the wild.

In summary, there appears to be no basis for concluding that, despite the primitive aspects of its anatomy, *Inia* occupies a lower behavioral level than the delphinoid whales. The fact that the platanistids appear to be phylogenetically older than the delphinoids and yet possess equivalent behavioral capacities suggests that there may have been strong selective pressure for a common basis of behavior early in the evolutionary history of the odontocetes.

Summary

Observations were made on two Amazon dolphins, *Inia geoffrensis*, captured in the Amazon River near Leticia, Colombia, in early March, 1956, and kept at Silver Springs, Florida. A young male lived 12 months in captivity and an adult male 15 months. An autopsy of the former revealed pieces of water-logged wood in the stomach, congestion in the lungs and an extensive hemorrhage in the brain. The large adult apparently died from severe bronchial pneumonia. Flukes *(Hunterotrema caballeroi)* occurred in the lungs of the small male and trematode eggs were found in its brain. The roundworm *Anisakis insignis* was present in the stomachs of two other *Inia* collected in the vicinity of Leticia.

The heart rate recorded in the captives under different conditions averaged 60 and 96 per minute. Two rectal temperatures obtained were 97.4 and 96.9°F. Organ weights of the small male are given, the brain weight/body length index of this individual being 88. A single erythrocyte count from a sample of blood taken from the abdominal cavity of a specimen shortly after death was 3,671,000 per mm^3.

In a small channel where they were initially kept, the dolphins generally swam idly about in one pool for a long interval, then made a leisurely excursion to another, often returning to the original pool in a short time. They were somewhat more active when placed in a pen in the main stream. Evidence indicated that the animals became habituated to their surroundings and were reticent to leave them. Scratching on the bottom or on protruding objects was a commonly observed activity. The dolphins evidenced no well-marked diel activity cycle. In the channel, the dolphins apparently slept on the bottom for brief periods. An apparently sleeping individual observed in the pen in the main spring floated off the bottom, with the body forming an S-shaped curve and only the tail in contact with the substrate.

The normal swimming speed was between 1 and 2 m.p.h., and the maximum speed recorded was approximately 10 m.p.h. The reduced dorsal fin and large, flexible flippers appear to be correlated with the relatively slow swimming speed. The general mechanics of locomotion are typically cetacean. In slow swimming a slight lateral movement of the tail stock was noted on occasion. This component was more pronounced when the dolphins were turning. The head is very mobile, being moved more during slow swimming than fast. The dolphins frequently performed barrel-rolls while swimming or from a position of rest and often swam upside down. Observations also suggested the occurrence of echelon-formation swimming.

Inia typically surface to breathe in a horizontal position, although they roll in more typical dolphin fashion under some conditions. In normal surfacing, the blowhole remains open for approximately 2 seconds. The mechanics of the blowhole operation appear to be similar to those described for delphinids. Intervals between blows ranged from 18 to 130 seconds. The small male tended to respire more often than the adult. There seemed to be a tendency for the dolphins to alternate a series of short intervals between breaths with ones of longer duration, although the pattern was not clear-cut.

Although the eyes are small, vision in *Inia* seems to be normally developed. As in other cetaceans, hearing is exceptionally acute and is probably the chief source of information about the environment. Circumstantial evidence suggests the existence of active echolocation in this species. The long beak appears to have an important function as a tactile organ, and the numerous, bristle-like hairs present on the beak probably contribute to this function.

The captives were quick to react to changes in their environment and showed a strong inclination to avoid strange objects under water. Without special training, they learned to respond to a splash as a feeding signal in a few days and in

three months' time had become quite tame. The younger individual was more curious and tamer than the adult. It would take food directly from the hand and allow a person to ride on its back.

The stomach of a wild *Inia* contained mostly fish remains (Characinidae), although a seed, insect fragments, and a small amount of vegetation were also present. The captives were fed dead fish. On only one occasion was a captive seen to capture and eat a live fish (gar). The pair consumed about 20 to 35 pounds of food per day. Fish under a foot in length were preferred. The prey was typically grasped with the tips of the long jaws then passed to the rear and masticated thoroughly before being swallowed head first. The dentition of *Inia* is obviously specialized for its particular manner of feeding. The anterior teeth are suited for grasping and holding and the molariform posterior teeth for mastication. The nature of the rear teeth suggest that in the wild *Inia* may take hard-bodied prey. The behavior of the captives and the specialization of the beak for probing suggest that *Inia* often feeds on the bottom. In comparison with other platanistids, *Inia* appears to resemble *Lipotes* most closely in its general feeding habits.

The dolphins frequently indulged in sexual behavior. Sexual activities recorded included masturbation, swimming with erections, attempted intromissions with one another and attempted copulation with inanimate objects.

Both animals engaged in considerable play activity. Individual play consisted of toying with sticks, pieces of vegetation and dead fish. Sticks were sometimes thrown out of the water and retrieved. The most common joint play activity was the passing of sticks back and forth as the dolphins swam around. This continued even after the animals were separated by a fence, the sticks being passed from one animal to the other through the meshes of the fence. The young individual was observed on several occasions engaging in behavior that suggested he was attempting to entice the larger individual to chase him. After five months in captivity, the small male would approach a human in the water and try to initiate play by clasping the person's hand against its body with a flipper and attempting to swim off.

The large male uttered squealing notes during the flight from South America. Sounds heard by observers under water included high-pitched squeals and a sharp, loud percussive note accompanied by clapping of the jaws. Under some conditions, a loud snort, produced by forced exhalation, appeared to represent some sort of communication, perhaps an alarm or warning signal. Two types of vocalization were recorded with a hydrophone.

During the first month in captivity, the two dolphins remained in close association at all times, the smaller individual taking the initiative in maintaining contact. Only one aggressive act was observed during this period. After this time the pair stayed together less and agonistic behavior increased, the large male being the aggressor in all observed instances. By the fall of 1956 it became necessary to separate the two animals, as the large one almost continually harassed the smaller.

Comparison of the behavior of *Inia* with data available for the bottle-nose dolphin and other small marine odontocetes suggests a basic similarity in the behavioral capacities of these forms. The fact that the platanistids are structurally more primitive than the delphinoids may indicate a strong selective pressure for a common behavior pattern early in the evolutionary history of the odontocetes.

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EXPLANATION OF THE PLATES

Plate I

Fig. 1. Adult male *Inia.* Note poorly-developed dorsal fin, large flippers, elongate rostrum, and conspicuous melon. (Photograph by Bruce Mozert, Silver Springs, Florida).

Fig. 2. Adult male and young male swimming together during early period of captivity. The position of the small male suggests the possibility that this activity represents echelon-formation swimming.

Plate II

Fig. 3. Young male leaving surface after breathing. (Photograph by Bruce Mozert, Silver Springs, Florida).

Fig. 4. Adult male breathing. Note the way in which the large melon deflects water away from the open blowhole.

Plate III

Fig. 5. Adult and young male breathing in synchrony.

Fig. 6. *Inia* seizing dead fish at surface.

Fig. 7. Adult male masticating fish at rear of jaws before swallowing.

Plate IV

Fig. 8. Jaws of an old adult female, showing worn and broken teeth in anterior part where prey is usually grasped.

Fig. 9. Young male trailing adult in channel.

Fig. 10. Adult male and young male turning while swimming together. Note more prominent crest on the caudal peduncle of the adult.

FIG. 1

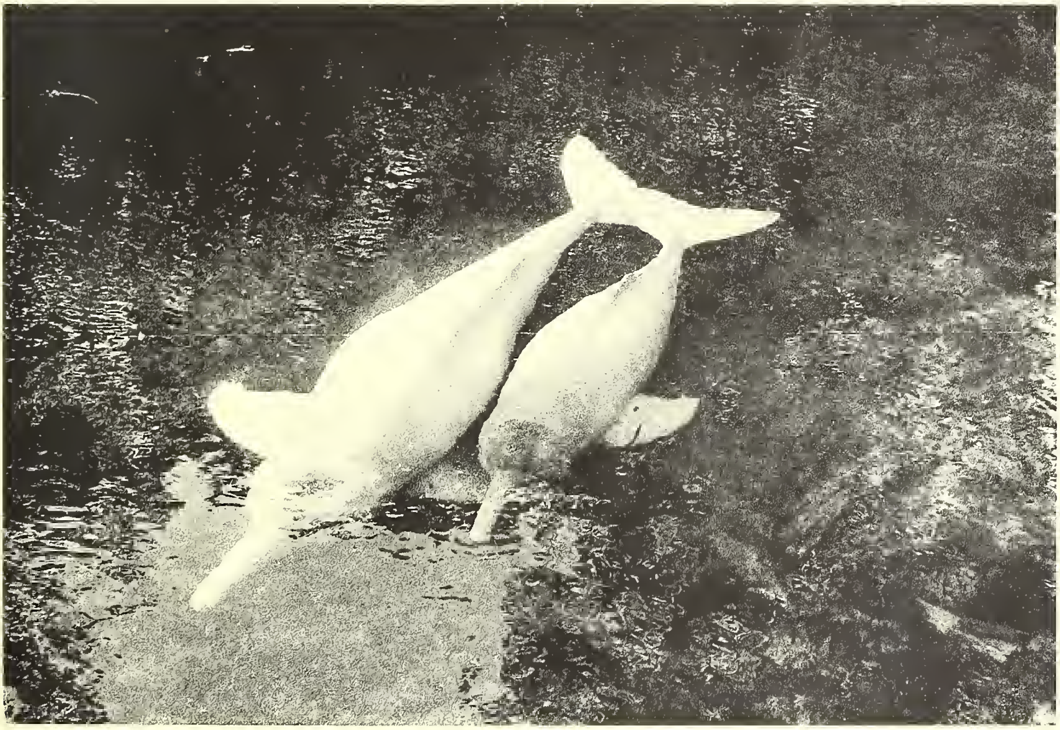

FIG. 2

BEHAVIOR OF THE AMAZON DOLPHIN, INIA GEOFFRENSIS
(BLAINVILLE), IN CAPTIVITY

FIG. 3

FIG. 4

BEHAVIOR OF THE AMAZON DOLPHIN, INIA GEOFFRENSIS
(BLAINVILLE), IN CAPTIVITY

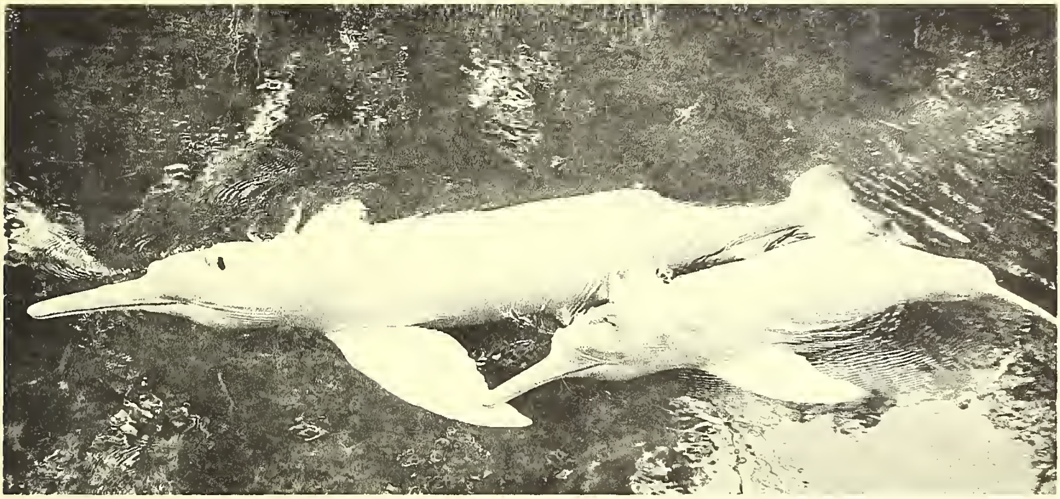

FIG. 5

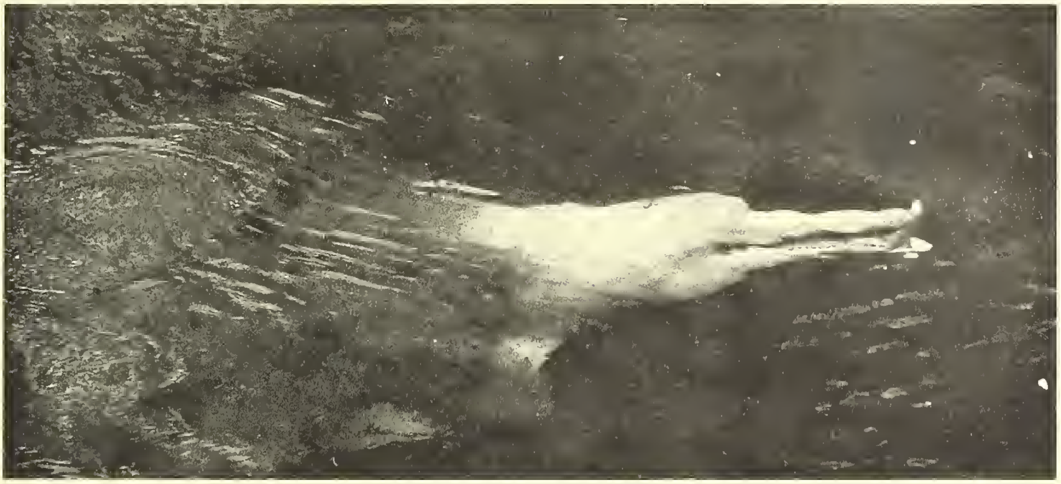

FIG. 6

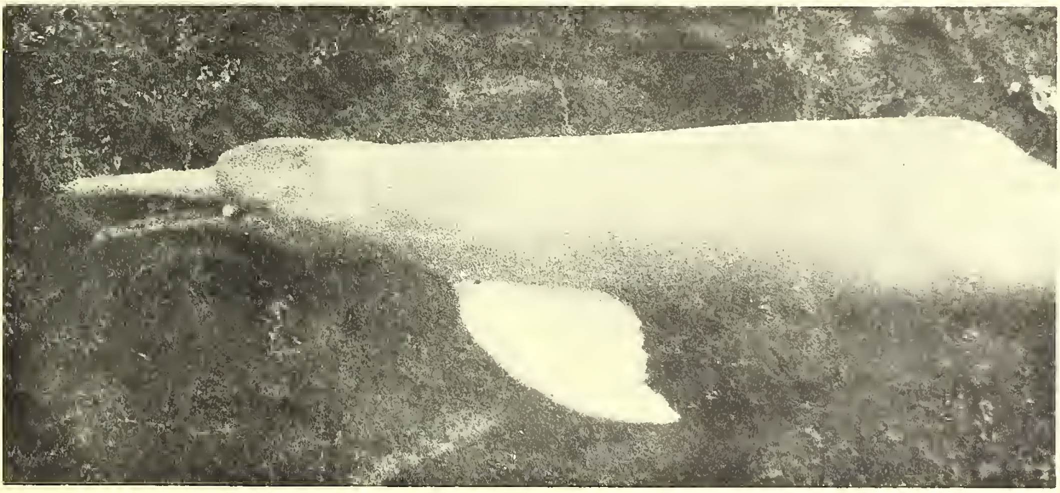

FIG. 7

BEHAVIOR OF THE AMAZON DOLPHIN, INIA GEOFFRENSIS
(BLAINVILLE), IN CAPTIVITY

FIG. 8

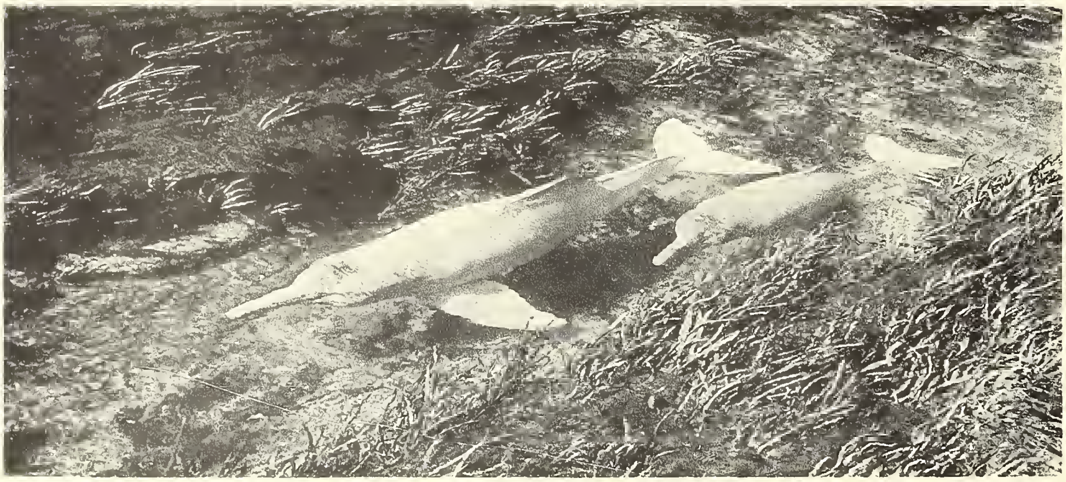

FIG. 9

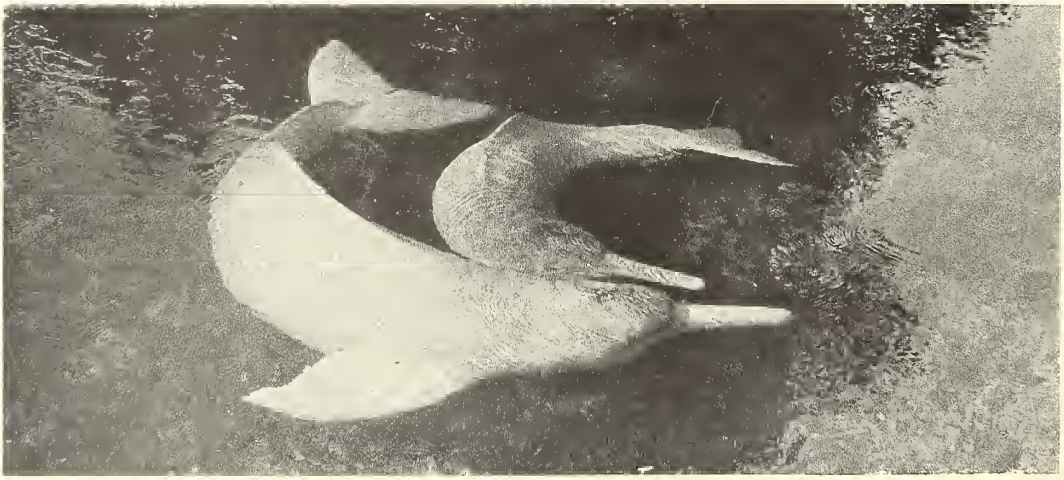

FIG. 10

BEHAVIOR OF THE AMAZON DOLPHIN, INIA GEOFFRENSIS
(BLAINVILLE), IN CAPTIVITY

6

The Development of Curiosity Within the Genus *Panthera*

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(Text-figures 1 & 2)

RESPONSE to novel objects, frequently taking the form of play, is one of the most striking behavioral characteristics of the infant mammal. Moreover, it is generally accepted that such reactivity reaches a peak during the early life of the animal and then declines during adult life. As Beach (1945) has indicated, however, there has been little systematic data on which to base this belief, primary support for the view being provided by a wealth of anecdotal lore (Welker, 1961). In one of the first experimental approaches to this question, Welker (1956) examined the development of curiosity in the chimpanzee and substantiated the existance of a period of maximum reactivity followed by a reduction in magnitude of response.

The present report concerns an analysis of the ontogeny of curiosity in a group of zoo-reared cats. Our procedure has been adapted from the one previously employed by Welker (1956).

METHOD

Subjects.—Twenty-two infant cats were tested including: 3♂ and 2♀ lions *(Panthera leo)*, 4♂ and 2♀ tigers *(Panthera tigris)*, 3♂ and 2♀ leopards *(Panthera pardus)*, 3♂ and 2♀ jaguars *(Panthera onca)*, and 1♀ snow leopard *(Panthera uncia)*. Two of these animals, a lion and a tiger, had to be dropped from the final sample when they became excessively disturbed during the tests. All of these animals were born at the Lincoln Park Zoo in Chicago between 1960 and 1962. It is standard procedure at the zoo to remove baby cats from their mothers at birth and rear them in incubators. Surviving members of a multiple birth are reared together, barring the development of some infection. Therefore, these animals generally had considerable contact with a member of their own species, as well as with their keepers.

An additional sample of adult cats, including 2 tigers, 2 leopards, and 2 jaguars, was tested at the New York Zoological Park during the summer of 1961. One male and one female of each species was tested. All had been born and reared in the zoo. The 2 tigers were approximately 4 years old and the 2 jaguars approximately 12 years old, while the male and female leopards were 16 and 3 years old, respectively.

Apparatus.—The following objects were used to evoke responses from the infant cats: (1) Two wooden "blocks," including a 12-in. length of 1 × 2 and a 6-in. length of 1 × 1; (2) Two pieces of steel chain, including a 12-in. length of #210 tensile chain and a 6-in. length of single jack chain; (3) Two wooden dowels, including a 12-in. length of ⅝-in. dowel and a 6-in. length of ⅜-in. dowel; (4) Two pieces of black rubber tubing, including a 12-in. length of ½-in. tubing and a 6-in. length of ¼-in. tubing; (5) A crumpled piece of white bond paper, 8½ × 11 in.

The objects used with our adult sample were similar to the above, but were scaled to a larger size, except for the crumpled paper which was of the same dimensions as that used with the infant animals. (1) The wooden blocks consisted of a 24-in. length of 2 × 4, and a 12-in. length of 1 × 2; (2) The pieces of chain included a 24-in. length of #20 welded chain and a 12-in. length of #210 tensile chain; (3) The dowels included a 36-in. length ⅞-in. dowel and a 12-in. length of ⅝-in. dowel; (4) The two pieces of rubber tubing were 24 in. × 1 in. and 12 in. × ½ in.

With few exceptions, each stimulus object was used with but a single cat, in order to avoid residual influences of prior tests. The exceptions all occurred with the large chain used with the adult cats. As we were unable to obtain sufficient new chain of this type, we were forced to reuse some lengths. However, all such pieces of chain were carefully washed before reusing.

These objects were similar to those used in a previous study (Glickman, Sroges & Hoff, 1961) and were chosen to provide a wide range of textures, odors and shapes.

Procedure.—All animals were tested either in their home cages or in an identical cage to which they had been habituated prior to testing. Testing was carried out between 5 and 10 p.m. when the zoo was closed to the public.

Each stimulus set listed above was placed in a subject's cage for a single 6-min. test session. At the end of the 6th minute, the stimulus set was withdrawn and, after a lapse of 10 minutes, the next stimulus set was placed in the cage. Objects were always presented to the cats in the following order; wooden blocks, chains, dowels, rubber tubing and crumpled paper.

Responses were scored by the experimenter on a sheet ruled into 72 squares. Each minute was divided into twelve 5-sec. periods. If an animal either oriented toward an object or touched it, a notation was made in the appropriate square. Contacts with objects which occurred without orientation, *i.e.*, those apparently made accidentally during the animal's movement about the cage, were not scored. If an animal both contacted an object and examined it visually simultaneously, only a contact response was scored. Thus, the maximum score, if an animal responded to an object throughout a 6-min. session, was 72. In the later tests, a record was kept of the animal's mode of contact with the objects and the vigor of its responses.

Efforts were made to retest all of our infant cats. Unfortunately, it was not possible to schedule retests at regular intervals and in some cases second tests were never obtained. However, we ultimately were able to test three subjects on three occasions, and ten subjects on two occasions. The remaining seven cats were given but a single test.

All adults cats were retested after a 4-wk. interval.

RESULTS

Quantity of Response in Infant Cats.—In order to provide an over-all view of the relationship between age and quantity of response, mean response scores were computed for each animal. These mean values were derived by summing the total response scores to the different test stimuli and dividing the result by five. Each of these mean scores was then entered as a single box in the appropriate column of Text-fig. 1.[1] A total of 10 tests between 4-6 weeks, 17 tests between 8-13 weeks and 6 tests between 15-24 weeks are represented in this bar graph. Inspection of this graph reveals a systematic increase in response from the first to the third age group, with all age groups differing significantly from one another ($p < .05$).[2] There is no overlap in scores between the first and third groups.

Analysis of data from repeated tests supports the above result in nearly every case. Eight subjects were tested during both the first and second age periods. Six of these animals increased markedly in mean response during this time, while two animals showed slight declines, giving a mean increase of 18.1. Six animals were tested both during the 8-13 week period and the 15-24 week period. All of these animals showed increased mean scores, with an average increment for the group of 29.0. The single snow leopard was first tested at 9 wks. of age, obtaining a mean response score of 68.4. Retests were carried out at 14 and 52 weeks, at which times the mean response scores were 53.6 and 71.5. This snow leopard was highly reactive from the earliest tests and displayed a full range of chewing, swatting and leaping responses to the objects.

The trend toward increased response with increasing age, among the infant animals, occurs with all five test stimuli (Table 1).

The sample size is too small to permit assessment of possible species differences in rate of development; however, reference to Text-fig. 1 indicates a fairly even distribution of scores within our age categories for all four species. In the only case where statistical comparison is feasible, the five 8-13-week-old tigers (mean response score = 42.2) do not differ significantly ($p > .10$) from the five jaguars in the same age group (mean response score = 36.6).

Habituation in Infant Cats.—There is no evidence for a significant decline in response with continued exposure of the infant cats to the test stimuli during the 6-min. test sessions (Text-fig. 2). The apparent trend toward habituation in the 4-6-week group is due entirely to the behavior of two leopards who were quite responsive at the beginning of each test session, but fell asleep toward the close of the session.

Categories of Response in Infant Cats.—Our literary protocols, detailing the modes of reaction used by infant cats, contain only general,

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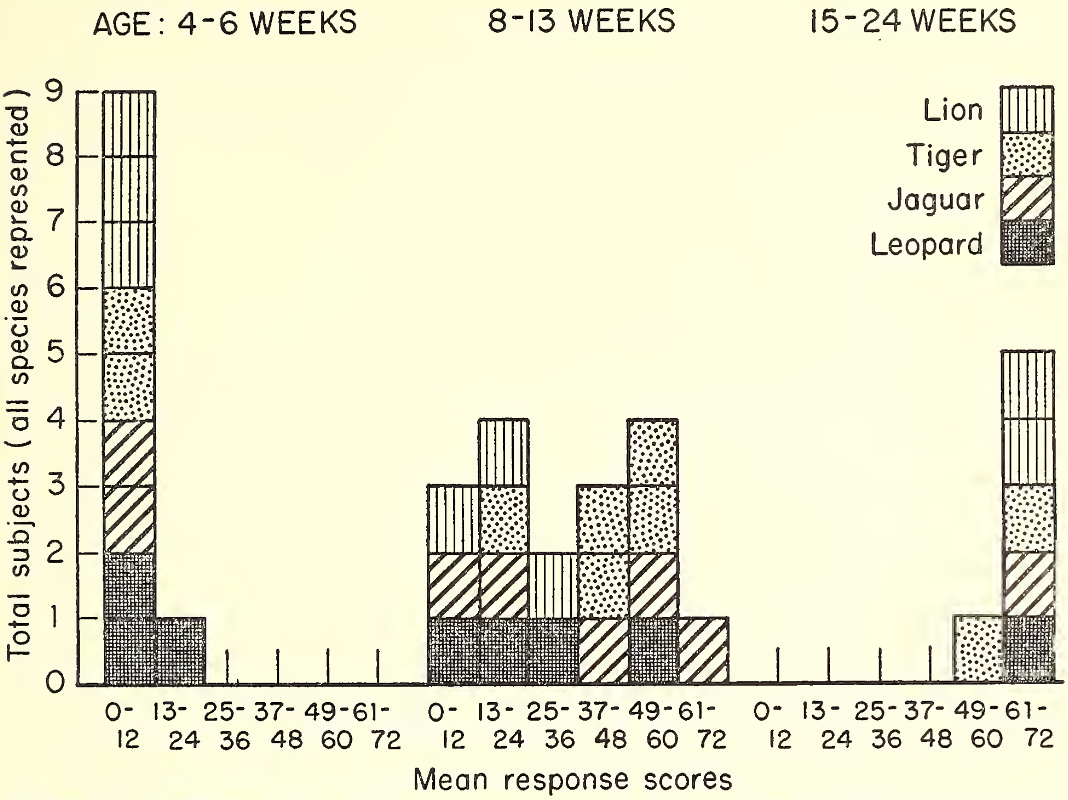
[1] The results obtained with the infant snow leopard are omitted from this and all subsequent tabulations, as only one representative of this species was available.

[2] Wherever a significance level is reported in this paper, the Mann-Whitney U-test (Mosteller & Bush, 1954) constituted the statistical procedure employed.



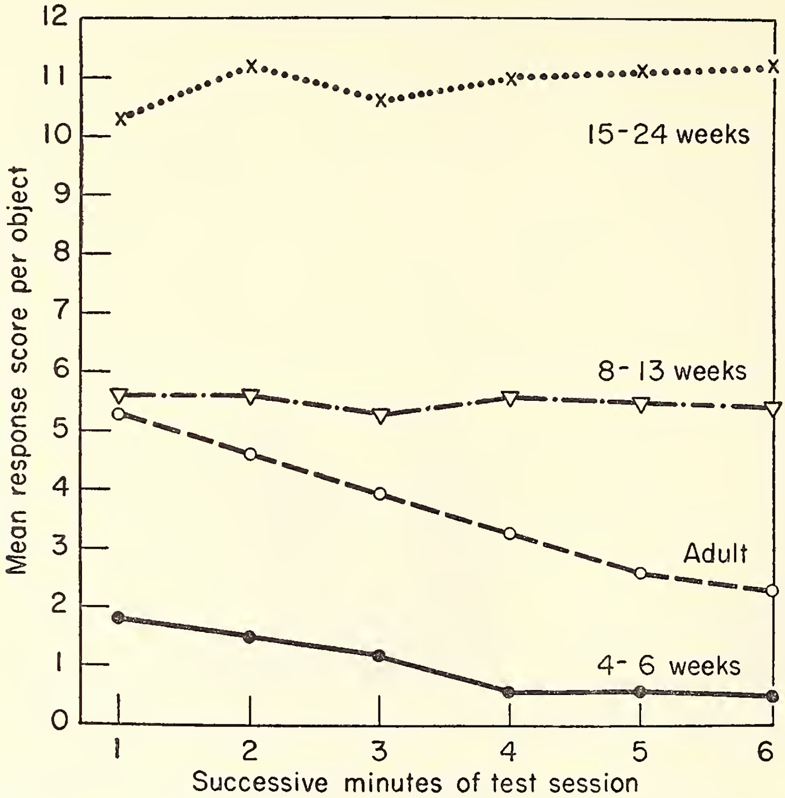
TEXT-FIG. 1. Mean response scores for individual animals according to age and species. Each box represents the mean response score of a single subject of the appropriate age group and the species indicated in the legend.

qualitative statements about the observed behavior. However, we have compiled the reactions of our cats according to six response categories for each age group. An animal simply had to show a particular reaction for a single 5-sec. period in order for that reaction to be appropriately tabulated; however, in most cases the tabulated reactions occurred much more frequently than that. Although our data are too scanty to permit statistical analysis, the following age trends are worth noting (Table 2): (1) A decreased number of cases where the subject falls asleep during the tests, and (2) increments in chewing, use of forepaws and vigorous play. The basic orienting response to the introduction of an object-set consisted of turning the eyes toward the object, approaching it and sniffing. This is evidently a relatively primitive reaction. It appears in animals within all of our age groups; however, only in some cases is it followed by sustained manipulation with mouth and paws.

Quantity of Response in Adult Cats.—The reactivity of our adult group is presented in Table 1 for each set of tests. It will be observed that the mean increment upon repeated testing, after a four-week interval, was 6.8. This increment was not significant ($p > .05$) and is much smaller than the increments observed with the infant groups, suggesting that increased response with increasing age, among our infant groups, was not due to any simple effect of repeated tests.

TABLE 1. MEAN RESPONSES TO DIFFERENT STIMULI ACCORDING TO AGE GROUP

Group	Blocks	Chain	Dowel	Tubing	Paper	Mean Total
4-5 weeks	9.3	3.2	5.1	2.6	8.7	5.8
8-13 weeks	38.8	31.9	22.1	30.7	35.0	31.7
15-24 weeks	58.8	65.3	69.0	69.8	67.2	66.0
Adult (test 1)	45.3	5.3	20.0	41.5	1.8	22.8
Adult (test 2)	39.0	6.2	36.2	65.0	1.2	29.5



TEXT-FIG. 2. Mean reactivity to novel objects as a function of continued exposure during test sessions.

Whether one considers the first or second set of tests, our adult sample differs significantly, in terms of mean total response, ($p < .05$) from the 15-24-week group.

Habituation in Adult Cats.—All six adult cats were less responsive in the sixth minute of the test sessions than the first (Text-fig. 2). Thus, our adult animals differ from our infant group in this respect.

Categories of Response in Adult Cats.—Our records of individual reactions are somewhat more detailed for the adult sample than for the infant groups. We have tabulated these separately for each adult cat (Table 3). The material presented in Table 3 represents a combined analysis of both the initial test and the retest. All activities checked occurred on at least two separate occasions.

Sniffing responses occurred in all of our adult cats, usually during preliminary investigation of an object. Further manipulation with the mouth and forepaws would usually, but not invariably, follow such an olfactory reaction. Simply steadying the object with the forepaws while chewing was the most common use of the limbs, but swatting the objects was also quite common. Frequently, after initial examination, the object was carried to a corner of the cage, or to a rear shelf, where vigorous manipulation ensued. Four of the six animals rolled over on their backs while manipulating the objects with forepaws or mouth. This behavior sometimes included using the hindlegs to kick at the object, or holding the object in the teeth and pushing against it with the forepaws.

Some of our subjects interrupted examination of the objects to pace the front of the cage, or to engage in characteristic stereotyped behavior, *e.g.*, rubbing the head against the bars of the cage (the ♀ tiger), or swatting the door of the cage (the ♂ tiger). Although none of our adult cats were observed to actually fall asleep during these tests, all of these subjects at some time during testing sat or reclined in the rear of the cage, or on the shelves which lined the rear wall.

DISCUSSION

These results substantiate the common views concerning the development of high reactivity toward novel stimuli during the early months of life, with a subsequent decline in total response during the adult years. Such ontogenetic changes in reactivity parallel the development of play as

TABLE 2. PERCENTAGE OF SUBJECTS EXHIBITING VARIOUS BEHAVIOR CHARACTERISTICS TO AT LEAST ONE SET OF OBJECTS

Behavior	4-6-week group	8-13-week group	15-24-week group
Falling Asleep	50	23	0
Sniffing	70	69	not available
Chewing	20	85	100
Use of Forepaws	50	92	100
Vigorous Play	0	69	100
Fear	20	16	0
(Total Subjects)	(10)	(13)	(6)

observed by Cooper (1942) in the captive African lion. Cooper suggested that play increased in lions from the age of one month to approximately one year, but noted that such behavior decreased in frequency from that point and was "conspicuously infrequent" in five-year-old lions. Our results further suggest that this decline is the result of both a decrement in initial reactivity and a faster rate of habituation in adult animals. However, it should be noted that when our adult animals did react, there was frequently great richness in the reaction patterns, with perhaps even more variety of response than in the quantitatively more reactive younger animals.

The reaction patterns observed in these cats, including the use of mouth and forepaws, were strikingly similar to those analyzed by Leyhausen (1956). As Leyhausen has indicated, all of these reactions can be related to the normal prey-catching and feeding repertoires of these species. It is interesting to observe that these basic reaction patterns appeared in a group of cats that were entirely zoo-reared and, in the case of the infant animals, deprived of any maternal example. These findings also coincide with Cooper's (1942) observation that the play responses of hand-reared lion cubs were quite similar to those of maternally-reared cubs.

TABLE 3. NUMBER OF ADULT SUBJECTS EXHIBITING VARIOUS BEHAVIOR CHARACTERISTICS TO AT LEAST ONE SET OF OBJECTS

Behavior	Initial Test Sessions	Retest Sessions
Sniffing	6	6
Chewing	6	6
Carrying in Mouth	5	4
Shaking in Mouth	1	0
Licking Object	2	3
Holding in Paws	6	5
Swatting with Paws	5	4
Leaping at Object	0	2
Rolling on Back	3	3
(Total N)	(6)	(6)

SUMMARY

Responses to novel stimulus objects were studied in a variety of infant and adult cats of the genus *Panthera*. Our results indicate a gradual development of reactivity in these species, with a subsequent decline in over-all level of response. However, in those cases where adult animals did react, a considerable variety of response patterns of great vigor were observed.

ACKNOWLEDGMENTS

The infant cubs observed in this study were tested at the Lincoln Park Zoo in Chicago, Illinois. The authors are indebted to Mr. R. Marlin Perkins, Zoo Director, and the Chicago Park District, for their cooperation. The adult animals were tested at the New York Zoological Park and we are also grateful to Mr. William Conway, Zoo Director, and Dr. Herndon G. Dowling, Curator of Reptiles, for providing permission and facilities for testing. Finally, we wish to acknowledge the crucial assistance of Mr. K. E. Hartz and Mr. W. Renner in testing the infant cats at the Lincoln Park Zoo, and to Mr. Fred Martini for his assistance with the adult cat tests at the New York Zoological Park. The research was supported by a grant from the National Science Foundation (G17496) and this paper was written during the senior author's tenure as a Miller Fellow at the University of California, Berkeley.

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7

Manatees in the Guianas

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INTRODUCTION

THROUGHOUT the world the Sirenia are disappearing, and the two genera which remain are all that are left of a once widespread order of mammals (Allen, 1942, pp. 528-552). In recent times a third genus, *Hydrodamalis,* the large Steller's sea cow of the Bering Sea, was completely exterminated by irrational killing, probably within thirty years of its discovery in 1741 (Stejneger, 1887, p. 1054).

The dugong *(Dugong dugon),* at present found in small numbers around the tropical and subtropical coasts of the Indian and west Pacific Oceans, is the only remaining species of the Family Dugongidae which fossil evidence shows to have been formerly almost world-wide in its distribution. Manatees (*Trichechus* spp.) now live in tropical and sub-tropical rivers and coastal waters on both sides of the Atlantic. They belong to the Family Trichechidae for which so far there is no fossil history. Simpson (1932, p. 498) suggests that the manatee may possibly have started as a fluviatile form in West Africa and then spread to America, thus replacing the earlier dugongs in the Atlantic.

There are now three species of manatees, *T. senegalensis* in West Africa, *T. manatus* in the Caribbean and Gulf of Mexico, and the smaller *T. inunguis* in the Amazon and Orinoco Rivers. *T. manatus* is divided into two subspecies, *T.m. latirostris* around the Florida peninsula and *T.m. manatus* in the rest of the region, including the Guianas. There seems, however, to be some doubt about the validity of any anatomical distinction (Moore, 1951a, pp. 17-18).

The survival of both dugongs and manatees is now precarious and both are in need of proper conservation. Both are large, slow-breeding, defenseless animals with palatable flesh. Both are already much less abundant and less widely distributed than formerly. It was in an attempt to help towards their conservation that we went to British Guiana early in 1962 to study the distribution and habits of manatees. The work was made possible by a generous grant from the New York Zoological Society, followed by others from the Nuffield Foundation, the Zoological Society of London and Cambridge University (Foreign Travel Fund). British Guiana was considered to be the best place in which to study manatees as it probably contains as large a stock of these animals as any country. Further, the presence there both of helpful people and of manatees in a state of semi-domestication made possible the best use of the three months available.

A preliminary letter and a short paper dealing primarily with the status of manatees, their present abundance and possible economic uses, have already been published (Bertram & Bertram, 1962, 1963). The present paper deals mainly with more detailed observations on the habitats and habits of manatees in the Guianas.

DISTRIBUTION AND ABUNDANCE WITHIN THE GUIANAS

The shore is so shallow and the water so laden with silt carried down by the Orinoco and other huge rivers that the sea in this northeastern region of South America is brown in color, turbid and almost devoid of fixed vegetation. Thus it is a most unpromising environment for manatees, which are entirely herbivorous animals, normally grazing on "sea grass" and other aquatic plants. Nevertheless, manatees are found in the sea all along the Guianan coast, but it is probable that they are traveling from one river to another, rather than living a permanently marine life. They are most often seen near the mouths of rivers or in the neighborhood of sluices ("Kokers") guarding the outflows of drainage channels from the sugar plantations. Both rivers and channels may carry floating vegetable matter into the sea. No evidence exists as to whether manatees are directly affected by the salinity of the water in which they live.

All the main rivers of British Guiana and of Surinam (formerly Dutch Guiana) probably contain manatees in the flat coastal regions. The absence of a coastal plain in Cayenne (French Guiana) results in there being few suitable habitats in that country. The passage of manatees far upstream is in many cases barred by rapids, but in some rivers, particularly in regions of wet savannah, the animals probably live along the whole length. The areas providing the best environment, the largest masses of floating grass, are the Courantyne River region on either side of the eastern boundary of British Guiana and the rivers of the extreme northwest of the country. Further west is Venezuela, which still contains considerable stocks of manatees.

Though it is comparatively easy to ascertain the distribution of manatees by traveling around the country and questioning the local people, it is far more difficult to find out about the abundance. Direct observation is virtually impossible. Not only are the rivers extremely large, the Essequibo being over 20 miles wide at the mouth, but the water is dark brown and usually opaque with mud; the manatees are invisible apart from the chance glimpse of the tip of the nose when breathing. The only figures available (and none of them are surrounded by sufficient evidence to give them any exactitude) are these: in 1959 in nets tied across the piers of the bridge across the Canje River, nine manatees were caught in two months; in 1962 during our visit five were brought into Georgetown from the Abary River and the catcher said he had eight more fenced off ready for capture[1]. Probably a total of between 70 and 100 individuals have been caught for the Fisheries Service in these two rivers since April, 1959.

Apart from that information, present distribution and frequency data have to be built up from such reports as: "In some of the rivers of the northwest, you will probably see a manatee every half hour when traveling by canoe;" "There were two feeding on this corner last Saturday;" "I have never seen one;" or "Manatee meat appears in this market about once in two months." Integrating such material, we arrived at the tentative conclusion that there are now probably some thousands, but not tens of thousands, of manatees in British Guiana. It is certainly the general belief that they are much scarcer than in former times and have been scared away from many of the lower reaches of rivers by the increasing use of powered boats.

AVAILABILITY

The availability of manatees in captivity made it possible to study their habits in some detail. Not only were there partially tamed animals in the public pools in the Botanic Gardens in Georgetown, which would in the evenings take grass from the hands of visitors, but there were also newly-caught individuals in more private and in more extensive waters. Under a valuable scheme initiated by the Fisheries Research Officer, Mr. W. H. L. Allsopp, in 1959, manatees are obtained by the Fisheries Service, kept for a while to make certain that they are healthy and then passed on to the Department of Drainage and Irrigation for use (Bertram & Bertram, 1963) in weed clearance in certain waterways. The possibility of handling these animals as they arrived, and of being able to watch their behavior at leisure, gave much useful information, unobtainable under natural conditions. Further, two of these manatees were living in the large reservoirs of the waterworks, which was the only place in British Guiana where the water was sufficiently clear for the animals to be visible when submerged.

SIZE

Early travelers[2] in British Guiana reported seeing manatees of immense sizes, up to 18 to 20 feet in length. Now they are believed not to exceed 11 to 12 feet with 7 to 9 feet being the commonest. According to the records of the Fisheries Service, of 33 individuals, presumably a random sample, the lengths ranged from 3 feet 5 inches to 11 feet. The weight of the smallest animals was recorded as 60 lbs. while the largest was over 1,000 lbs. Details of the measurements are shown in Table I below, but these are not sufficiently numerous or reliable to show whether there is any significant sexual disparity in size.

MOVEMENT

Manatees are most active in the evening and in the early morning. At these times they are feeding and occasionally disporting themselves vigorously in the water, almost like seals. They can swim quite fast, and when speeding it seems that they keep their noses above the surface. They usually travel totally submerged, making surprisingly little disturbance in the water. Swimming is by vertical strokes of the large tail. The power of this organ can be gauged by the size of the swirl and the violence of the thrashing when the animal is suddenly frightened or caught in nets, and by the many stories of capsized

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[1] These were later said to have escaped.

[2] e.g., Henry Bolingbroke (1799-1806) and Lt. Thomas Staunton St. Clair (1806-1808). Both these have recently been republished by the "Daily Chronicle," Ltd., Georgetown.

Table I. Lengths of Manatees Measured by Fisheries Service, British Guiana.

Length	Under 6 ft.	6-7 ft.	7-8 ft.	8-9 ft.	9-10 ft.	10-11 ft.	No. measured
Females	1	5	4	5	2	3	20
Males	1	—	5	5	2	—	13
	2	5	9	10	4	3	33

Detail Measurements of Three Manatees.

Measurement	Male	Female	Female
Length	7′ 0″	7′ 2″	7′ 8″
Girth	4′ 10″	5′ 1″	5′ 4″
Length of tail	1′ 7″	1′ 11″	2′ 0″
Width of tail	1′ 7″	1′ 8″	1′ 9″
Length of flipper	11″	11″	1′ 2″
Length, nose to flipper	1′ 3″	1′ 5″	1′ 3″

canoes and broken backs when fishermen have come in contact. The flippers also help in swimming, particularly when the animal is moving gently about while feeding. They probably enable it to turn in its own length and to swim backwards without altering the symmetry of the body. The flippers help also in pushing the body up out of the water as far as the shoulders, with the result that the animal can graze on plants growing about a foot or more up river banks. Manatees are generally believed to be unable to leave the water, but there is some evidence that they can climb out onto dry land and can cross a low bank from one piece of water to another[3]. Certainly they are just able to wriggle back into the water if laid on a suitable bank nearby.

During the middle of the day manatees seem normally to be quiescent and often lie at the surface apparently basking in the sun. They may lie horizontally with the head visible, but more often the back is strongly arched and the head below water. If disturbed they are able to submerge, without noticeable movement and without a ripple or bubble of air appearing. Usually on these occasions the nose breaks the surface and a breath is taken shortly after the animal has disappeared. Occasionally manatees roll over on their backs when resting, but this may only be when they are sick. They seem to be gregarious by nature, for whenever several are kept together in enclosed waters, they tend to feed and to rest together. Furthermore they often breathe all at the same time, several noses appearing almost simultaneously and then disappearing again. Older evidence indicates a degree of gregariousness in the wild, and even recent stories refer to "schools" or groups being seen in several places. In Florida the other subspecies is reported by Moore (1956, pp. 1-10) to live in aggregations.

Movements, of the nature of migrations, may occur but present evidence is quite insufficient for assertion or denial.

Identification of Individuals

The desirability of identifying individuals in any biological study needs no argument. Though some manatees in British Guiana could be recognized by such features as the loss of an eye, or extensive algal growth on the skin, these peculiarities could, because of the opacity of the water, only be seen when the animals were lying at the surface with part of their bodies exposed. Attempts were therefore made to mark individuals, especially the tips of the noses, so that they could be more easily recognizable. Aluminum paint and some special red and yellow dyes[4], which had successfully been used on elephant seals in the South Orkney Islands, were tried. Unfortunately the skin of manatees seems to be continually sloughing off so that the dye is rapidly removed. Animals painted in this way were indistinguishable after two days except perhaps by examination so close as to be impractical.

Neither branding nor tagging were tried, the

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[3] The former Curator of the Botanic Gardens, the late Mr. G. E. Wolstenholme, said he once put a manatee in a channel separated from another channel by 2 feet of dry ground and that during the night it crossed from one piece of water to the other.

[4] Four dyes were kindly supplied gratis by Imperial Chemical Industries, Ltd. They were Auramine O, Rhodamine B, Methasol Fast Yellow R and Methasol Fast Red B.

dangers of fungal infection being high in these waters[5].

BREATHING

Normally manatees keep their nostrils closed, whether they are above or below the surface, and only open them when a breath is taken. When basking or feeding, the only occasions when it is possible to be certain that the same animal is being watched, the nostrils seem to open at intervals of about one to two minutes; eight recordings giving figures from 55 seconds to 2½ minutes. Certainly they can hold their breath for very much longer periods than this and Parker (1922, p. 133) has shown that for the other subspecies a figure of 16 minutes, 20 seconds has been recorded. Scholander & Irving (1941, p. 183) found that manatees submerge with their lungs full of air.

Both exhalation and inhalation are normally quite silent. The nostrils are usually open for under two seconds so that the volume exchange must be small. Only on one occasion, when an animal was startled, was there a sound of a breath being taken[6]. This, apart from the splashing, was the only noise they were heard to make in British Guiana, although one traveler in British Honduras[7] said that he heard a manatee give "a deep, hoarse bellow, not unlike a foghorn."

The capability of manatees for vertical motion without horizontal movement presumably depends upon muscular compression or relaxation of the lungs with consequent change of density, for such movement can take place without expiration or inspiration.

FEEDING

Manatees are voracious herbivores and consume large quantities of soft vegetable matter. They seem catholic in their tastes and will eat a great variety of species whether they be in the sea, in freshwater or on the river bank, and whether they be rooted on the bottom or floating on the surface. Texture seems more important than type of plant, which may range from fine grasses to leaves of "mocha-mocha" *(Montrichardia arborescens)*, and they will take any plant that is sufficiently soft to be torn off by the muscular upper lips. These lips, strengthened by lateral horny pads and armed with hairs, bristles and fibrous papillae, grasp and tear the vegetation with a movement reminiscent of the mandibles and proboscis in some polychaete worms. The food is then passed back to the grinding molars which are the only teeth present in the adult. These teeth, as in the elephant, move continuously forward during life, the individual tooth starting at the back of the jaw and eventually falling out at the front.

Sometimes the flippers are used to sweep the food towards the mouth, but at others the animals seem to rest on their finger tips and grope among the weeds with their snouts.

AWARENESS

A large herbivore perhaps does not need very keen senses for survival where food is abundant. The eyes are small, but directed upwards. It would seem that this position might be effective for an animal feeding on the surface of the water, but would preclude any binocular vision. Sight is probably not a well-developed sense and no difference in behavior was noticed in an individual which had lost its right eye.

Hearing, on the other hand, seems to be remarkably acute, despite the absence of external ear and the very small size of the auditory passage[8]. It is common practice locally to whistle to attract the attention of manatees living in semi-domestication and they appear to respond equally well when submerged or when the head is above water.

Careful observation of newly-caught manatees suggests that hearing is more important than sight in the detection of the presence of man, the chief enemy. On thirteen occasions it was possible to approach basking manatees to distances between 4 and 12 feet and they would take no notice even if their eyes were visible and open. If a hat was then waved in front of them, as on four occasions, there was still no reaction. However, on all these occasions the animals submerged immediately if the observer spoke. That manatees are particularly aware of

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[5] All manatees caught by the Fisheries Service were treated orally with sixteen 250 mg. tablets of Griseofulvin as a fungicide.

[6] Vincent Roth, formerly Director of the Georgetown Museum, reports hearing a "long blast of air from its wide nostrils" in his "Notes and Observations on Animal Life in British Guiana," 1941, published by the "Daily Chronicle," Ltd., Georgetown.

[7] Thomas Gann, Ancient Cities and Modern Tribes; Duckworth, London, 1926 (pp. 25-29).

[8] Prof. R. J. Harrison and Dr. J. D. W. Tomlinson, of the London Hospital Medical College (University of London), in describing the dissection of a 6′ 3″ specimen from British Guiana, write:

"The external auditory meatus is a very narrow tube that scarcely admits a seeker. It is about 40 mm. in length and at the surface only 2 mm. in diameter. The meatus is a fairly straight tube, narrowing to almost occlusion point where it obliquely meets the tympanic membrane. The inside of the meatus is a pigmented greyish colour and there is no sign whatever of any wax plug . . . The tube is embedded in semifluid mucoid tissue and as it approaches the tympanic membrane becomes almost undissectable."

human beings is supported by the fact that such actions as stamping, clicking the tongue, starting a motion picture camera or whistling, either naturally or with a variable-pitch dog whistle, evoked no response, whereas the human voice, however soft, was immediately effective and the animals submerged. Perhaps in the muddy waters of British Guiana it is not surprising that ears rather than eyes seem to be used in detecting danger. It is probable that vibrations in the water are also keenly perceived. There is no information about their powers of smell, though their olfactory organs are anatomically well developed. Manatees are believed by some to possess a homing instinct and to be able to find their way back to their original river from considerable distances[9].

BREEDING

Very little is known about the breeding of manatees in British Guiana. Despite exhaustive enquiries, only three people were found who had seen them mating (twice in the Abary River and once in the Botanic Gardens in Georgetown). On all three occasions the animals were in shallow water. The most detailed account states that at 9 a.m. on January 9, 1955, about thirty-five miles up the Abary River, a school of 14 to 16 manatees was watched for 2½ hours and "they were disporting themselves and gave the impression of fighting. Later they moved into the shallow and worked themselves up the bank into six inches of water. Then they mated lying on their sides." The river was said to be in flood and two feet above the normal level[10].

It may well be that the availability of precise physical circumstances will prove important in attempts to get manatees to breed in semi-domestication.

No information is available about gestation periods, details of birth, suckling habits or rate of growth of this subspecies. Of the Florida subspecies at least one manatee has been born in captivity, and there is evidence that gestation is more than 152 days (Moore, 1951b, pp. 26-29; 1957, pp. 137-138).

Surprisingly, among all the many people questioned in British Guiana, only eight had seen young manatees, though admittedly it is difficult to judge the size of these animals in their natural conditions. These young were reported from various parts of the country and there seemed to be no proper deduction that one area was better than another for breeding. Among the animals which had been kept in the Botanic Gardens since 1885, at least one birth had taken place, but not in the last twenty years. Evidence here suggests that manatees are long lived, but there are insufficient records to give actual ages. It is certainly evident that they are very slow breeders.

Attempts to rear on a bottle two young ones caught at sizes of 3 ft. 5 in. and about 4 feet were unsuccessful and the animals both died within a month of capture.

BALANCE IN NATURE

In considering the possible future of manatees, it is well to consider the factors, both disadvantageous and advantageous, influencing the survival of the species. In British Guiana and probably everywhere, the main enemy is man. Not only does man kill manatees for meat, but he disturbs them with his guns and propellers. The expanding population brings greater disturbance and the increasing use, in particular, of outboard motors on dugout canoes, is undoubtedly scaring these animals away from many of the lower reaches of the rivers.

Lesser enemies than man are the caiman, which may possibly take a small toll, especially of young animals. Parrot-fish, too, would be likely to devour any injured specimens. Apart from their large size, manatees are defenceless creatures with neither offensive teeth nor claws. Not only are the individuals vulnerable to any attack, but the stock as a whole is easily damaged because of the slowness of their breeding.

Factors favoring survival fortunately also exist. Probably most important of these are the surreptitious habits of the animals themselves. Their acute awareness of danger from human beings and their silent submergence must give them good natural protection. In addition there is legal protection. In British Guiana no manatees may legally be killed except by occasional special permission from the Director of Agriculture. Unfortunately it is always harder to enforce legislation than to make it, and particularly is this so in a country of poor communications and a population short of meat.

Possibly the most important favorable factor is the potential use of manatees as weed clearers. In limited areas, making use of their large appetites and varied tastes, Allsopp has shown them to be extremely efficient at clearing weed-choked channels and in keeping clear the water in pools and waterways (1960, p. 762). So far, none of the manatees thus used has bred under condi-

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[9] Mr. David Richardson of Booker Bros. writes that "on two separate occasions, a manatee got out from the channel in which it was penned and was found sixteen miles away, but prevented from getting into the Canie River (from which it had come) by a sluice."

[10] This information was communicated to Mr. Arthur Goodland of Booker Bros., who kindly forwarded it.

tions in which they work in weed clearance so that this use at present constitutes a further drain on the stock. However, if this difficulty can be overcome, and manatees can be brought to breed freely in this state of semi-domestication, then there is great scope for expanding this use. Once a species becomes of economic value, its chances of survival increase.

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8

Self-contained Diving Operations in McMurdo Sound, Antarctica: Observations of the Sub-ice Environment of the Weddell Seal, *Leptonychotes weddelli* (Lesson)

CARLETON RAY[1] & DAVID LAVALLEE[2]

(Plates I-III; Text-figures 1-5)

INTRODUCTION

DIVING with self-contained underwater breathing apparatus (SCUBA) has proved to be a valuable scientific tool, permitting the investigator to probe personally into the underwater environment. Its principal use has been in temperate to tropical littoral waters. As scientists gain experience in diving technology, its use is spreading farther afield.

During October and November, 1963, the New York Zoological Society sponsored an expedition to McMurdo Sound, Antarctica, for a study of the physiology and ecology of the Weddell seal, *Leptonychotes weddelli* (Lesson). At that location at that time of year, the Sound is covered with an almost solid layer of 2-4 m. ice. A significant part of our investigation involved submarine observation with SCUBA under the sea ice to know firsthand the conditions in which the seal exists and to record the sound emissions which seals may use for navigation and/or communication. Sound production by phocid seals has been the subject of a preliminary study by Schevill, Watkins & Ray (1963) and Weddell seal sound production and its possible significance have been described by Ray & Lavallee (in manuscript) and Schevill & Watkins (in manuscript).

Techniques of polar diving are very different from those used in warmer waters, and much of the known information has not been published. Fane (1959), Neushul (1961) and the U. S. Navy Diving Manual (1963) discuss some techniques of cold-water or polar diving, but none treats the special problem of diving under solid sea ice. Articles in diving magazines have dealt with the similar, but not so rigorous, subject of sub-ice lake diving. Therefore little is known about the reliability of open-circuit breathing apparatus or of the physiological effects on divers under field conditions under sea ice in locations such as Antarctica.

The diving program was divided into two phases, based on the advice and publications available. The first was to test materiel and observe physiological phenomena on divers using open-circuit lungs in a preliminary effort to establish possible endurance limits. The second phase was to make observations and photograph the sub-ice environment. Testing was carried out to determine: (1) performance of valve blocks and regulators; (2) exposure protection and buoyancy problems of the diving suit; (3) physiological problems such as vertigo, loss of orientation, nitrogen narcosis threshold, low temperature torpidity and cramps; (4) reliability of photographic equipment, battery lamps, depth gauges and surface-supplied floodlights.

ACKNOWLEDGMENTS

This program was supported by Grant GA-57, Office of Antarctic Programs, National Science Foundation, to the New York Zoological Society, and by the Commander, U. S. Naval Antarctic Support Force. Some physiological equipment was supplied through Grant AINA 76 from the Arctic Institute of North America to the New York Zoological Society.

Considerable help prior to our expedition was received from personal communication with Dr. M. Neushul of the University of California at Santa Barbara and from Mr. Verne Peckham of Stanford University. Additional aid and ad-

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[1] Associate Curator, New York Aquarium, New York Zoological Society, New York.

[2] Lieutenant, U. S. Navy, Headquarters, Third Naval District, New York.

vice were given by Lt. Commander Glenn Brewer, U.S.N., of the submarine *USS James Monroe,* who dived in the Arctic Ocean under the ice. The sound and consistent advice of these men was extremely helpful in the selection of equipment and in planning and carrying out the dives.

Our field operations would have been impossible without the logistic support of the USARP office and personnel based at McMurdo and the help of Dr. Donald Wohlschlag and his assistants of the McMurdo Biological Laboratory. Mr. Elmer T. Feltz of the Arctic Health Research Center, Anchorage (a member of the expedition team), and Mr. Gerald Kooyman of the University of Arizona gave unstinted aid in field operations as did Lewis E. Roane, PH2, Billy D. Douthit, PH3, and Joseph B. Phillips, AN, of the Atlantic Fleet Mobile Photo Unit, U.S.N., Norfolk.

The following read and criticized this paper: Dr. Donald Wohlschlag, Dr. Michael Neushul, Mr. Verne Peckham, Dr. John Bunt of Sydney University and Dr. Laurence Irving of the University of Alaska.

MATERIEL

The following equipment was taken for the diving operations:

Neoprene exposure suit (wet type). See below for details.

Two sets of compressed air lungs. Twin 70 cubic foot (4000 L) steel bottles with "J" reserve air block, 2150 psi.

Two double-hose, 2-stage regulators (Aqua Master, U. S. Divers).

One single-hose, 2-stage regulator with pressure gauge (Sportsways Waterlung).

Weight belts, masks, flippers, depth gauges, diving watches, underwater knives, underwater flashlights.

Inflatable diving safety vests.

Underwater battery-powered sealed-beam lights.

Two 1,000-watt floodlights and cable, with spare bulbs.

One Xenon flashing beacon in watertight case.

Four 15-pound grapnels.

Four cork buoys.

Four small styrofoam buoys.

600 feet of ⅜-inch braided nylon line.

1,000 feet of nylon parachute cord.

One Navy-type diving stage (one man).

The selection of exposure suits was of particular concern. Mobility and a certain amount of comfort were desired. A combination of a wet suit with a dry suit worn over it would offer the greatest endurance, although bulky. Information on chemical, or electrically heated, suits was not available, if such devices are in existence. A ¼-inch (6 mm.) neoprene wet suit was chosen as the most practical in shallow water. Commander Brewer, Dr. Neushul and Mr. Peckham recommended this thickness from their experiences.

Parkways Fabricators of South Amboy, New Jersey, assisted in designing and tailoring a satisfactory suit. With some minor modifications this suit would be ideal for shallow water diving. The suit consisted of three major pieces. The ¼-inch (6 mm.) pants came up to the armpits in a modified "farmer Brown" design. A ⅛-inch (3 mm.) thick armless undershirt with attached hood was worn under a ¼-inch jumper with a separate hood. Zippers were eliminated to minimize water entry. The three-fingered gloves and the boots were made large enough to prevent restrictions of blood circulation. To increase the thermal insulation, a very low density neoprene was used; all components were lined with stretch nylon. The outer hood was designed to come low over the forehead to protect the sinuses. The bottom part had a chin cup, so that with the mask in place only a minimum of flesh was exposed to the water.

Other equipment and support to carry out the diving was supplied as an integral part of Base operations and consisted of:

Electrically driven, 3-stage Cyclone air compressor.

An insulated hut, 2.4 × 3.6 m. with a 1.3 m^2. hole in the floor.

Oil heater.

2.5 kw. gasoline generator.

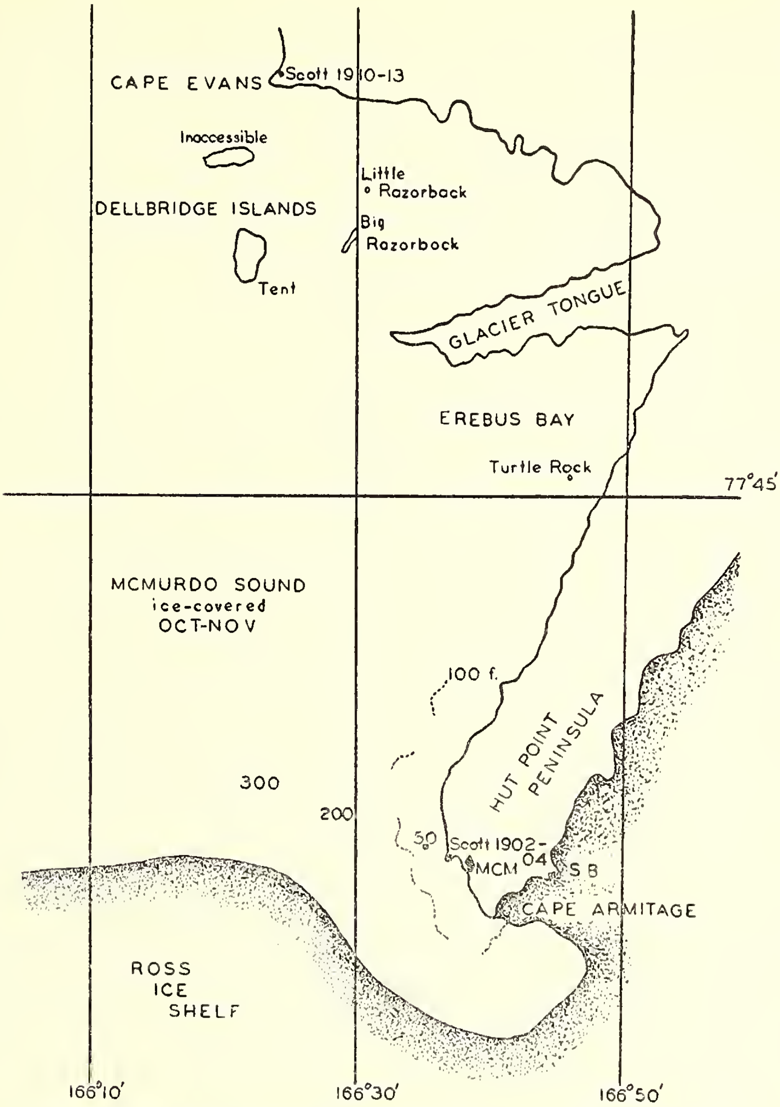
Diving ladder and strongbacks (for suspending the descending line and diving stage below the hole).

Coleman lantern, jerry cans, fuel, shovels, dipnets, ice chisels, chain saw, ice tongs.

Compressed air for the lungs was supplied by the air compressor installed in the Biology Laboratory. The intake for the compressor was extended to the outside atmosphere. This dry, cold air supply reduced the possibility of water accumulation through condensation and the resultant freezing at the regulator high-pressure stage during diving operations.

OPERATIONS

Preparatory.—A point was selected on the sea ice in McMurdo Sound, about a quarter of a mile offshore (Text-fig. 1). The weather was still quite cold, early Spring temperatures running —20 to



TEXT-FIG. 1. McMurdo Sound from Cape Evans to Cape Armitage. MCM = McMurdo Station (USA). SB = Scott Base (NZ). Soundings are in fathoms: 50 = location of diving hut. Redrawn from chart HO 6666.

−30°C with winds gusting to 40 knots or more on some days (Table 1). Water temperature under the ice was −1.9°C. Ice thickness varied from 2 to 3.5 m.

Text-fig. 2 and Plates I-III show our diving operation. A shelter was necessary from which diving operations could be conducted. This permitted divers to enter and leave the water in a warm atmosphere. First, a hole had to be cut through the hard sea ice. A 2 m². area was marked off and swept clear of snow. The ice was sliced into squares with a chain saw and then broken into blocks by stabbing between the cuts with a long-handled chisel. These cubes, weighing approximately 30 kg., were then lifted out with tongs. The process was repeated until a bottom layer of about 15 cm. remained. The ice removed from the hole had to be dragged some distance to distribute the weight. Past experience of scientists cutting holes in the thick sea ice had shown that ice removed from the hole and concentrated in an adjacent area caused drifting of snow. The combined weight of the blocks and snow pressed the ice down and water flooded over the edges of the hole. Usually, an ice sounding is taken by drilling a 1½-inch (4 cm.) hole in an adjacent area. When this was attempted, the bit became frozen in the ice and

TABLE 1. RÉSUMÉ OF DIVES MADE, WITH AMBIENT WEATHER CONDITIONS OBSERVED PARTLY BY THE AUTHORS AND PARTLY BY THE U. S. NAVY AT MCMURDO STATION.

No.	Date 1963	Time	Duration (minutes)	Air T° C	Hut T° C	Water T° C	Wind (knots)	Total Skycover %	Purpose	Depth (meters)
1	31 Oct.	1700-1720	20	−20		−1.9	20 E	60	Initial test	5
2	1 Nov.	late afternoon	20	−18	−2	−1.9	25-30 NE	30	Initial test	5
3	5 Nov.	1631-1656	25	−12	11	−1.9	5-10 E	50	Physiological	1
4	6 Nov.	1520-1550	30	−6	15 est.	−1.9	0	0	Testing gear	10
5	7 Nov.	1500-1520	20	−4	15 est.	−1.9	0	60	Observation of ice and seals	25
6	8 Nov.	1745-1810	25	−8		−1.9	14 E		Observation of ice	25
7	9 Nov.	1452-1526	34	−7	15 est.	−1.9	15 NW	30	Physiological	1
8	10 Nov.	2130-2200	30	−6		−1.9	14 SE	10	Observation of ice	10
9	11 Nov.	2330-2401	31	−4	15 est.	−1.9	8 NE	80	Physiological	1
10	12 Nov.	2000-2030	30	−4		−1.9	8 SE	60	Observation of ice and seals	12

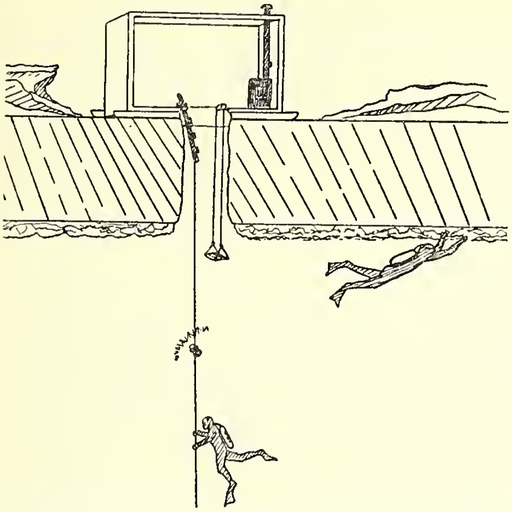
the operation was abandoned. Our not knowing the exact thickness of the ice slowed the cutting process considerably, and it was necessary to proceed with caution near the bottom. Once a hole was made through the final layer, water flooded over the edges of the hole. Usually 25 cm. of the top. After the hole had flooded, the final layer was chopped out with a long-handled chisel. The time required to clear the 2.3 m. hole was 32 man-hours of heavy labor. Techniques involving blasting considerably lighten this work load, but these were not used by us.

When the hole had been completed, a prefabricated heated hut, 2.4 × 3.6 m., was dragged into place. The hut had a 1.3 m^2. opening in the floor for access to the water. Two 10 × 10 cm. wooden strongbacks were put over the hole. From one a wooden ladder was secured with a descending line hanging 50 m. into the water, and from the other a diving stage was suspended to just below the underside of the ice (4 m.). The stage was to be used for the divers to place cameras and make last minute adjustments before proceeding under the ice.

An oil stove was installed for warming the hut. Under normal conditions, this heater provided a comfortable 15°C and kept the surface of the water in the hole from refreezing, but when the wind came up the internal temperature dropped to about 0°C, necessitating the partial sealing of the sides of the hut to the sea ice with snow. A portable gasoline generator, delivering 2½ kw., furnished power for the underwater floodlights and interior lighting.

Diving.—A summary of dives is given in Table 1. Diving was begun on October 31 when, after several days of "white out" and winds of up to 70 knots, the wind velocity dropped to about 20 knots and the diving team was able to conduct a short test dive to check the operation of the demand regulators and the effectiveness of the wet suits.

Dressing in the exposure suits was done at the Biology Laboratory at McMurdo, about a mile and one-half from the hut. Transportation to the diving site was by a heated tracked vehicle. The suits provided protection against the air temperature when supplemented with a parka, hat and mukluks. The remainder of the diving equipment was put on in the hut just prior to entering the water. Under the cold conditions of October-November, and particularly when the hut was not sufficiently sealed against wind, it proved to be almost impossible to prevent the formation of new brash ice at the surface of the hole.



Text-fig. 2. Diagram of diving hut and method of sub-ice operations. See text for full explanation.

This was cleared before each dive. Ice crystals were also continually formed on the undersurface of the ice early in the season and the entry and exit of divers freed these, which floated up into the ice-hole to thicknesses of up to about 1 m. This offered no particular problem except the impossibility of seeing up or down through the hole and partially obscuring the hole as well. A net was almost constantly in use by aides in clearing this brash ice. A method used by Biological Laboratory personnel involved hole warmers extended into the water to keep water temperatures in the ice-holes just above the freezing point.

Each dive was planned as to length, depth and purpose before entering the water. Descent was made by ladder and then to the diving stage just under the ice for rendezvous. Brash ice and crystals were cleared from around the bottom of the hole by hand by the divers upon rendezvous.

Orientation to the ice-hole was provided by the white nylon descending line, and the orange diving stage. Additional orientation was provided by a flashing Xenon beacon, attached at 10 m. depth on the descending line, which emitted a 250,000 candle power flash every 1 second. Sorties were restricted to 10 m. horizontally from the descending line. Additional distance would require a tending line.

After initial testing of diving equipment, dives were made for physiological purposes and to check visability, currents, floodlights and photographic equipment. Two 15-inch brass reflectors, each with a 1,000-watt bulb, were mounted on a four-foot wooden crossbar and trained to illuminate an area approximately ten feet away. The light array was buoyed with a cork float on a line about 2 m. long. By permitting the buoy to rest against the underside of the ice, the lights could be left unattended. Small styrofoam floats were strung on the heavy electric cable to compensate for its weight.

Comments on Equipment and Operations

Wet Suit.—One of the most critical items of polar diving operations is the exposure suit. Neushul (1961) has discussed the relative merits of wet and dry suits. The 6 mm. neoprene suit, worn in layers as described above, offers adequate protection, if there is no flooding. The biggest problem in this respect was water entry in the gloves, boots and down the neck of the outer jumper. The following modifications in the suit described are suggested: (1) the boots should be incorporated in the pants or have a long overlap in cuff and pants. It will be found a little difficult to get out of such boots but worth the effort for dry feet; (2) the three-fingered gloves should be very carefully fitted, for the slightest restriction in the fingers will impede circulation and cause numbness. The gauntlet part of the glove should be tapered and cut to reach just below the elbow to prevent leakage; (3) the undershirt should have a high neck with a detachable 3 mm. hood. The outer jumper should have an attached hood of 4.5-6 mm. material. This outer hood must be very carefully tailored to fit over the inner helmet for good seal against water entry. Neushul stated that no part of the face should be in direct contact with the water if diving was to be continued for extended periods. Peckham did not find this to be the case, nor did we, and we did not design the hood to completely cover the mouth with a slit or hole for the regulator mouthpiece as used by Neushul. That arrangement we thought might be overly cumbersome should the mouthpiece have to be removed and replaced, as happens at times. Bunt found any exposure to the forehead to be extremely uncomfortable and this was confirmed by us; (4) detachable flutter valves (non-return) may be fitted on the back of the outer helmet, the upper part of the jumper and at the top of each thigh to aid in the expulsion of trapped air upon diving, and provide a means for injecting warm water after surfacing and venting of the suit when undressing.

Rewarming.—Warm water and towels proved to be extremely effective in rewarming the diver. The face and hands were immediately doused and dried upon emergence from the water.

Fins.—Fins should be loosely fitted with heel "Y" straps to keep them firmly on the feet.

Weight Belt.—The weight belt must be secure, yet easily jettisoned if necessary. The buckle must be of such a size as to permit operation with heavy diving gloves. The large lever-type buckle that releases with one hand is the most desirable. On one occasion out of water, an attempt was made to release the weight belt without success. The buckle had frozen and the bulky gloves made its operation impossible.

Face Mask.—The glass should be tempered, as is usual for the best quality face masks. The skirts should be very pliable. The mask and hood should be fit within 1 cm. or less.

Tank.—Twin bottles are recommended for their increased operating time. Single bottles are more comfortable and have sufficient air for normal dives when the diver does not go over 20 m. However, when the diver depends upon one hole for exit he may find a 500 kg. seal or other obstruction in it when ready to surface. In such a case the diver might have to wait patiently until the animal has completed breathing. The twin bottles would allow longer endurance. Loss of orientation or rescue operations also demand longer potential diving time.

Reserve.—The ring on the end of the reserve pull rod ("J" valve type) should be at least 7 cm. in diameter to accommodate the gloved thumb. It is impossible to hook the gloved thumb through the rather small ring at the end of rods provided as standard equipment. This difficulty had been anticipated and a larger ring had been fashioned before diving.

Regulator.—Underwater, we found that breathing was normal and not noticeably different from any other dive in warmer waters. Ice formed on the high-pressure stage of the regulator without malfunction. While still rigged for diving and wet after ascent, both divers went outside the hut to expose the equipment to the —20°C air temperature. A regular rate of breathing was maintained on the apparatus to determine if the regulator would become blocked with ice. The block of ice on the back of the regulator increased in size but there was no resistance to breathing.

Diving Ladder and Stage.—An adequate means for entering and exiting the water is of great importance. Frequently, a diver returns to the ladder with numbed hands and feet, and it is difficult to get out of the water. A good method is the use of a fireman's type of ladder (Text-fig. 2 and Plate II). A diver can pull himself up by his forearms if the hands are numbed.

The stage is used as an accommodation for placing equipment. It should be in a basket shape so that it is not necessary to lash down anything placed on it.

Light Rigging.—Heavy equipment, such as the floodlights, must be as close as possible to neutral buoyancy. Buoyancy is a very difficult problem because it is constantly changing with the amount of cable that is paid out. Experience showed that a buoy, with considerable positive buoyancy, can be hard to handle. If lights are to be used some distance from the hole, the buoy should provide only a few pounds of positive support and should be on a short line 1 m. long at most. The equipment then can be released, if momentarily not needed, so that it will come to rest against the underside of the ice. However, buoying equipment with any lines can be of considerable disadvantage in maneuverability because buoying lines increase the possibility of entanglement. For instance, on one occasion some difficulty was experienced with the floodlights during a dive to take 16 mm. motion picture sequences. In order to position the buoy, it was necessary to pull up on the weight of the descending line. Several feet of cable had been paid out from above and hung in a bight below the divers. When Lavallee released the descending weight, it caught the cables and hauled him down. One of the lines became entangled in the supply hose of the regulator and pinched off the air. At this moment, a large bull Weddell Seal swam into the area and surfaced in the ice hole. It appeared for a moment that Lavallee would have to squeeze past the animal to get air on the surface. However, the hose came free, making this unnecessary.

Orientation.—Under the clear conditions of October-November, the white nylon descending line and diving stage could easily be seen from 10-15 m. and the Xenon light for much farther. The hole could not be seen horizontally from even 5 m. from just the ice, but could be seen from below at up to 25 m. In murky water or at greater distance, each diver must use a guide line.

Buoyancy.—Table 2 gives data on weights that were necessary to achieve neutral buoyancy. We define neutral buoyancy or inhalation, with slightly negative buoyancy after exhalation. Achievement of neutrality is one of the most frustrating problems inherent in almost all exposure suits. It can be a factor of considerable danger as well. For instance, the wet suit compresses with descent; it loses insulating capacity directly with loss in thickness and negative buoyancy increases. Thus, without a constant volume arrangement, the diver is neutral only for one specified depth—a depth which must be planned *previously* to each dive. A diver trim for 10 m. with 12 kg. weights, descending to 25 m., would be negative 8 kg. Few divers can swim with this much excess and, if the descending line were not immediately at hand, the alternatives would be to sink (and perhaps drown),

Table 2. Buoyancy Data on Divers Wearing Exposure Suits Described in Text. Weights Were Worn on a Belt About the Waist.

	Lavallee	Ray
Height	6 ft. 0 in. (182 cm.)	5 ft. 11 in. (180 cm.)
Weight	175 lb. (77 kg.)	165 lb. (75 kg.)
Waist	33 in. (86 cm.)	32 in. (64 cm.)
Type	Mesomorphic	Mesomorphic
	Positive Buoyancy	
Surface	35 lb. (16 kg.)	32 lb. (14.5 kg.)
10-30 m.	26 lb. (12 kg.)	25 lb. (11.5 kg.)
25 m.	10 lb. (4.5 kg.)	10 lb. (4.5 kg.)

or to drop weights, and/or to inflate a safety vest. In the latter case, a special problem occurs in sub-ice Antarctic diving. A vest would arrest descent but as the diver ascends and still cannot find the descending line, he will float up at accelerating speed to rise into the thick brash ice which is present. Finding a way back to the ice-hole against such positive buoyancy with a cumbersome inflated vest in the brash ice would be difficult or impossible. The use of a safety vest is not to be regarded as safe under ice. If weights are dropped, the suit itself provides more than enough buoyancy for ascent from depths of 50 m. or more.

Such buoyancy problems comprised the main reason for limiting all vertical movements to the descending line, grasping the line and testing buoyancy and only venturing from it horizontally after achieving neutrality. If a problem such as sudden vertigo were to hit a diver away from the line without neutrality, one possible result would be drowning should the second team member fail in recovery.

Teamwork.—A diving team is normally a pair, acting as one man. Under Antarctic conditions, we found it mandatory that the team be augmented by one or two surfaces aides. If two teams are to be used, these could be alternated to extend diving time in repetitive dives; the surface team would act as aides for the submerged pair. Aides are important because, dressed in thick suits and wearing heavy gear, the diver out of water or at the surface is clumsy in the extreme. Normal movements are further encumbered by fatigue and numbing due to cold. Surface aides serve to keep the ice-hole clean and to maintain warm water and supportive gear ready for immediate use.

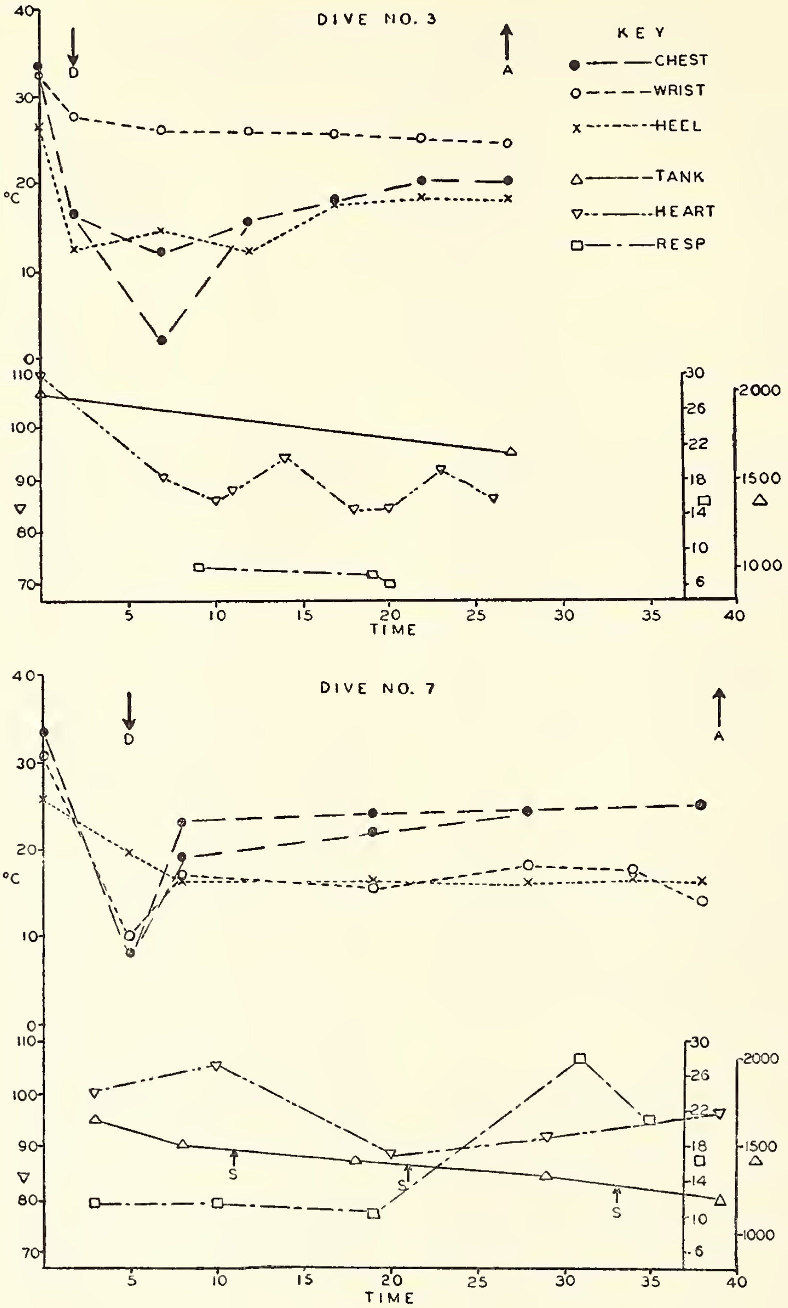
Mobility.—Dive number 8 (see Table 1) was made in an ice-hole a mile from the diving hut. We found no difficulty in dressing and loading as usual, moving the diving ladder with descending line and weight to the new location and diving. Mobile operations considerably farther from a diving base are perfectly feasible, as are field operations from a heated vehicle or helicopter.

Diet.—Caloric requirements (see "Physiology" below) are high during cold-water diving, at least 5,000 calories and possibly as much as 7,500 calories per day. If work is to be carried out far from a well-equipped base, food becomes a major item of logistics.

Time.—An entire dive, barring complications, approximated 2½ to 3 hours in time from dressing in the Laboratory to redressing there. Since the suits were excellent protection from ambient weather, considerably longer trips from base are perfectly feasible if adequate clothing and survival gear are also taken so that undressing could be done in the field. Neushul (1961) reports up to 6 hours' field operation dressed in a wet suit similar to the one we have described.

Two, or at the very most, three such dives might be attempted in a day, though it must be emphasized that each dive can entail considerable heavy work and even a two-dive day can be exhausting. Underwater diving time, as will be apparent under discussion on physiology, could perhaps be extended to hour periods provided that the diver remained active enough to generate sufficient heat to maintain the suit-skin temperature gradient over extended periods. Use of a larger hut than was available to us would save energy by eliminating the necessity of returning to the laboratory between dives.

Data Recording.—Both still and motion picture photography are advisable, though still black and white photography is simplest in the low light intensities encountered. Stroboscopic light is preferred because of the ease of operation without light bulbs and because of the speed of flash. For still photography we used the 35 mm. Calypso or Nikonos camera with 50-watt-second strobe designed by Harold Edgerton of The Massachusetts Institute of Technology. An excellent system of data-recording would involve

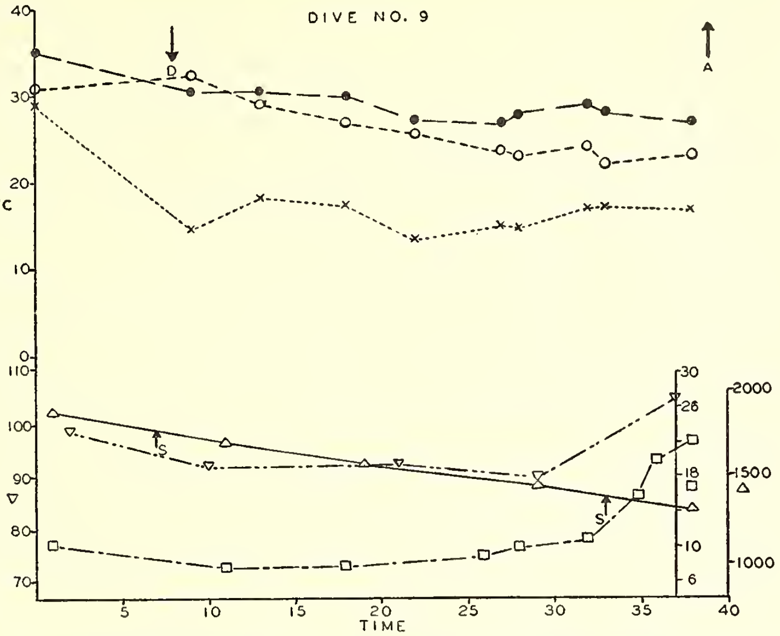


Text-fig. 3a **(Dive No. 3)** and 3b **(Dive No. 7)**. See text for full explanation. Times of entry and exit from water are marked with arrows. S= time sample exhaled air taken.

an underwater communication and tape recording facility. Several such systems have been designed (Breslau, Zeigler & Owen, 1962) and are available, but we have not field-tested any of them.

Physiology

Previous papers on the subject of polar diving have contributed to technology, but few physiological data have been gathered under field conditions. On dives 3, 7 and 9, Lavallee was the



TEXT-FIG. 3c **(Dive No. 9)**. See text for full explanation. Times of entry and exit from water are marked with arrows. S = time sample exhaled air taken.

subject of certain tests designed to establish possible endurance limits. The test dives were conducted in the ice hole with the diver hanging motionless on the descending ladder and with the head just below the suface. Results are shown on Text-figs. 3, 4 & 5.

Method and Equipment.—Skin temperatures were recorded on the bare chest, the ankle-heel region and the wrist-palm region by means of thermistor leads from a YSI Tele-thermometer inserted under the wet suit. Electrocardiograms were obtained with a battery-operated Cambridge Trans-Scribe with leads inserted under the suit to the ankles and wrists. Both the telethermometer and EKG wires caused some flooding into the suit, as is shown by temperature drops after immersion (Text-fig. 3), but since the subject remained motionless, further flooding did not occur and the water soon warmed so that it is improbable that there were significant heat losses. For instance, little flooding occurred on dive 9 and submersion time was not extended. Respiration rates were observed directly by means of the intermittant bubble stream from the regulator. A polyethylene tube attached to the regulator exhaust funnelled respiratory exhaust gases to the surface where they were collected with a syringe for transfer to a Scholander 0.5 cc. analyzer (Scholander, 1947). Continuous tank pressures, directly proportional to O_2 utilization, were read directly by means of the pressure gauge attached to the regulator during diving.

Dive 3, the initial test, was purposely shorter than dives 7 and 9. The latter two dives were carried almost to the limit of the subject's endurance.

The Subject's Observations.—The precedure outlined was extremely uncomfortable for the diver after about 20 minutes of submersion.

Lavallee reported two periods of cold stress on each of the test dives and these are confirmed by the data. One was just after submergence when certain flooding occurred and limited amounts of cold water came into contact with the skin. The second occurred after 20 minutes and increased thereafter. During the last 10 minutes of each dive, the extremities became painful and/or numbed. The subject was aware of greatly increased respiratory rate and shivering during the terminal stages of each dive, especially numbers 7 and 9. His impression was that another 10 minutes in the water might have been impossible.

At terminus of dive 7, Lavallee reported that the "hands were dead due to lack of circulation," that "spasms and a sick feeling" were experienced, and that he had had "difficulty in breathing, particularly on exhalation. It was as if I had indigestion and I felt little pains or kinks at the sides of the throat near the clavicle." It is difficult to say what caused these symptons, but they appear to be assoicated with the increased respiratory rate observed.

During the mid-stages of dive 9, Lavallee voluntarily increased the rate of respiration and reported feeling warmer as a result, probably due to the increased muscular activity of respiration. This is not in contrast with the observations on dive 7, above, because during the initial minutes of a test dive the subject is in better control of respiration than at terminus when shivering and respiratory rate increase more or less involuntarily.

Upon emergence from each test dive, warming was rapid. The subject reported feeling "normal" within 5 to 10 minutes and expressed willingness to dive again.

It was not possible to test adequately such problems as susceptibility to vertigo, loss of orientation, nitrogen narcosis threshold, and low temperature torpidity and cramps as originally planned. However, some observations were made. During one dive to 25 m., Lavallee's suit flooded up under the jumper. After a very few minutes he became rather uncomfortable and suspected that duration under such circumstances would have been about 10 minutes, which agrees with both Neushul's and Fane's comments. No cramping occurred. On another dive to the same depth after a purposely increased respiration rate to about 60 per minute. Lavallee began to ascend and experienced vertigo-like dizziness which disappeared after a pause at 12 m. on the descending line. Ray has experienced vertigo at 25 m. in water of 4°C. We cannot identify the cause, but possibly it is due to a sudden flooding of one of the ear canals with cold water or loss of CO_2 with hyperventilation. Vertigo is especially dangerous at depth under ice, since orientation is lost.

On another occasion, Lavallee pulled the outer hood chin cup down and away from the face and ears. Contact with water proved painful, especially around the chin and temples. The mouth and cheeks more easily tolerated the cold and the jaw muscles did not cramp. Vertigo was not experienced.

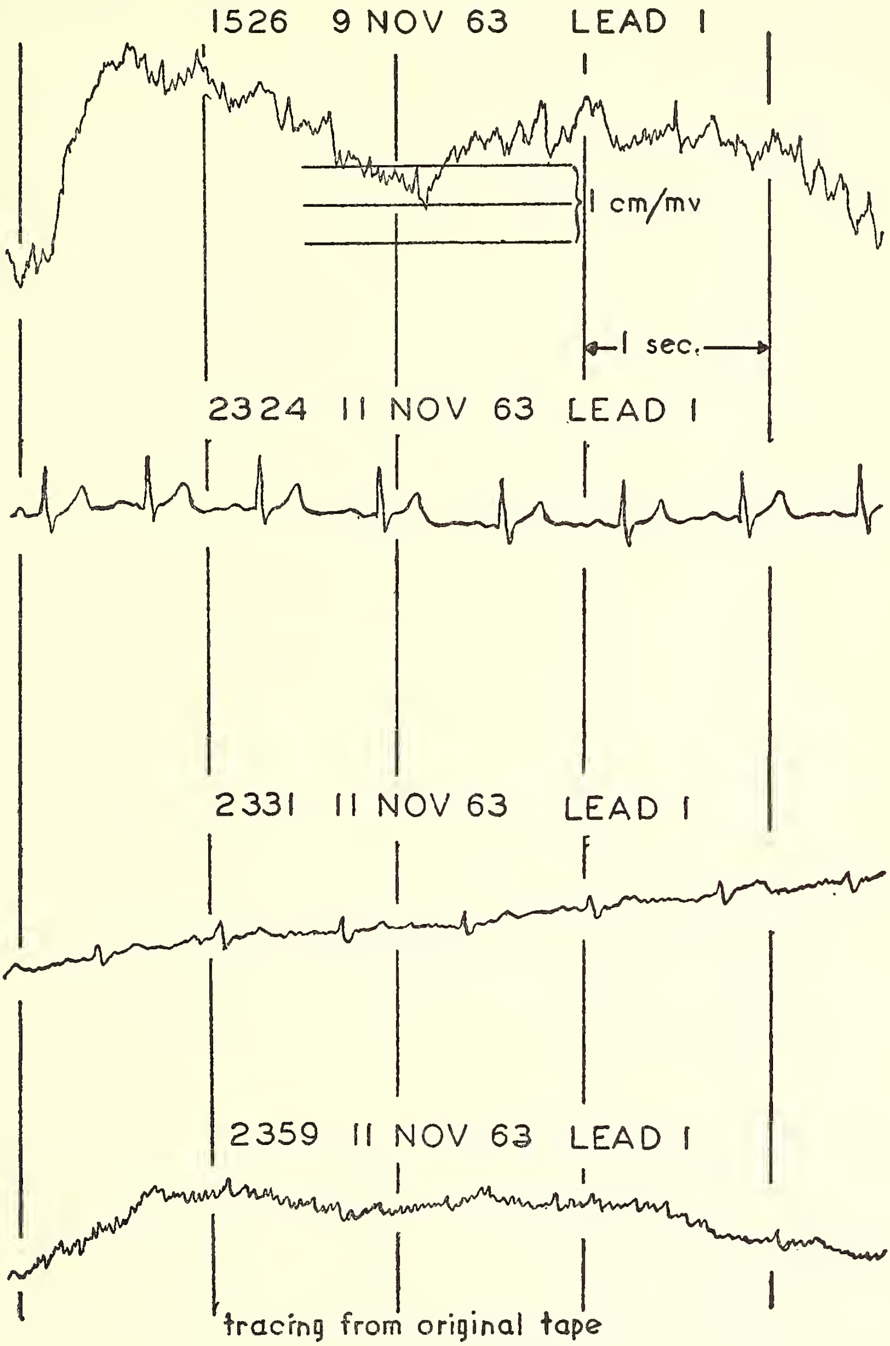
Skin Temperatures. — Temperatures of the wrist-palm, ankle-shin and chest are given in Text-fig. 3. In air of 24°C (about room temperature), Lavallee maintained normal resting temperatures while bare-chested, but wearing light pants, as follows: Chest 31°, ankle-shin 29°, wrist-palm 30°. Fully dressed in a wet suit just prior to entry on the test dives, these temperatures were 33-35°, 25-29° and 31-32° respectively. The elevated temperatures were due to activity and insulation of the wet suit. Immediately after immersion, depression in all these values was clearly but irregularly evidenced. The lowest temperatures recorded were 2°, 12° and 10° respectively. Thereafter, all temperatures rose as the water in the suit was heated by the body, then leveled off at about 20-30°, 14-18° and 18-26° and did not show terminal depression as might be expected. No temperatures were taken at the tips of the extremities and none internally.

Heart Rate.—Rates are shown in Text-fig. 3 and sample EKG's are given in Text-fig. 4. Due to shivering, and water interference, the EKG's could not be used for analysis of heart action, but rates are indicated. Lavallee's normal resting rate was measured at 72 and 80 on two occasions in the laboratory. No conclusive or consistent alterations of heart rate are indicated. Slowing from 100-110 to 85-95 after immersion was to be expected, since the work load was decreased. Several unexplained alterations in rates, perhaps due to alterations of breathing rate, are seen. Depression of rate or cardiac arrest, often associated with severe internal temperature depression, were not seen in any dive. Text-fig. 5 (a) shows the heavy shivering which is characteristic at terminus and is a good indication of involuntary metabolic adjustment being made in an effort to keep internal temperatures normal.

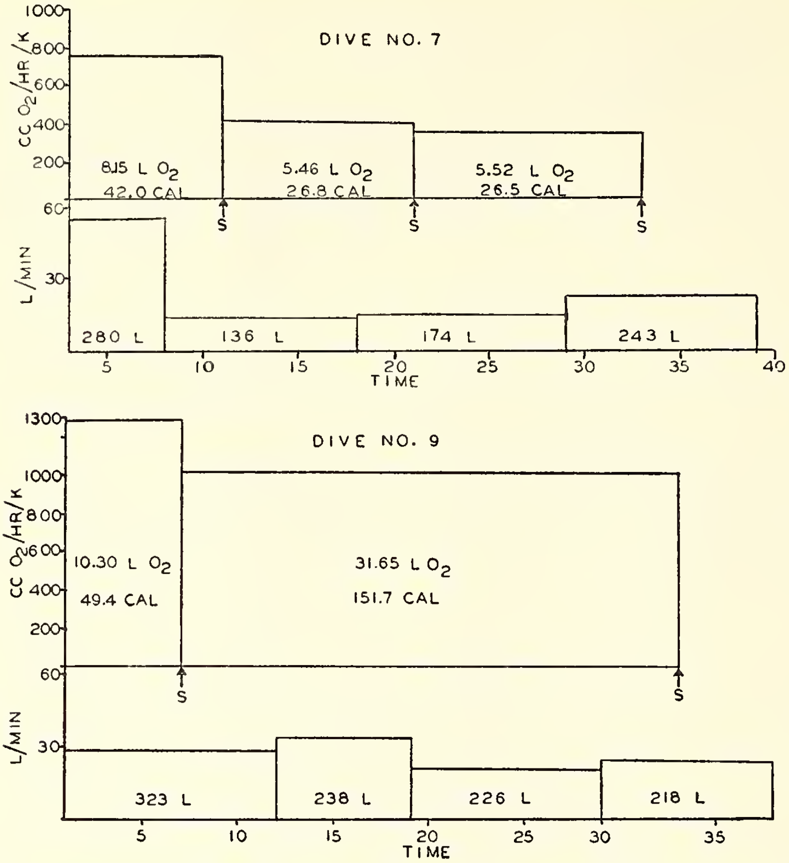
Respiratory Rate.—Certain unreliability must be attached to observed rates since these are partially under control of the subject. There is a tendency on the part of divers to unconsciously control the rate of respiration. Nevertheless, at termination, there is a strong increase in the rate of respiration, as shown on Text-fig. 3. This was associated with the diver's feeling cold and with shivering.

Metabolic Rates.—The regular fall in tank pressures on dives 7 and 9 is shown in Text-fig. 3. Details of metabolic analysis are given in Text-fig. 5. From tank pressure, the air volume consumed can be calculated and the results of dive 7 show what we believe is the more typical case of the two. Initial consumption was high, approaching 60 liters per minute. After adjustment to the water while remaining perfectly still on the diving ladder, the gas consumption fell as did the heart rate. At terminus, the rate picked up due to subject chilling and resulting increased respiration rate and shivering. Dive 9 did not show such dramatic variation, but both dives agree in the terminal stages in that the final consumption approached 25 liters per minute at the surface, which is 30% higher than our average in warmer waters.

Three gas samples were taken on dive 7 and two on dive 9. O_2 and CO_2 analyses differed for



TEXT-FIG. 4 **(A)**. Example of severe shivering, from an EKG taken 34 minutes after entry, Dive No. 7. Time is given by upper left number. **(B)**. EKG on surface before entry into water, Dive No. 9. **(C)**. EKG 2 minutes after descent, Dive No. 9. The impulse is not so strong, due to electrical interference of the water. **(D)**. EKG 30 minutes after descent, Dive No. 9. Shivering is evident.



Text-fig. 5a **(Dive No. 7)** and 5b **(Dive No. 9).** Air and O_2 consumptions during Dives 7 and 9. Lower blocks indicate volume of air utilized during the test period indicated. Numbers within these blocks give volume in liters. Upper blocks represent volumes of O_2 utilized. Numbers within these blocks give volume O_2 in the test period indicated and its equivalent in terms of Calories of energy expended. S = time of exhaled air sample (see Text-fig. 3). See text for full explanation.

the two dives. From these data, R.Q. and oxygen consumption have been calculated. These data are more revealing than air consumption since O_2 utilization was not equal throughout. Gas consumption was initially high in both dives in this case, and in spite of increased respiratory rate the O_2 consumption did not rise at terminus, indicating that increased respiration alone cannot increase respiratory efficiency. In all samples but one, the R.Q. was near 0.8. Initially on dive 7, the R.Q. was 1.02. Caloric utilization has been calculated using 5.15 Cal. per liter O_2 at R.Q. = 1.0 and 4.8 Cal. per liter O_2 at R.Q. = 0.8. The total calories utilized in terms of O_2 consumption on dives 7 and 9 are 95.3 and 201.1 respectively. These are widely divergent values, but both agree in being extremely high for a man at "rest," which is the state approximated after the initial "work" stage of each dive. The lowest Cal./min. values on each dive were 2.21 and 5.7 respectively. The highest values, during the work stage before and just after entry; reached 5.25 and 8.25 respectively.

Physiological Conclusions. — Man possesses few adaptations for the conservation of heat. His only defenses against cold are shivering, voluntary muscular activity and whatever insulation he may be able to devise. Witwer (1958) states that in water of 0°C, naked man's survival time is less than 1 hour. Seals immersed in freezing water allow a gradient to occur in which the skin temperature falls to near zero whereas the muscles a few inches under the thick blubber are nearly 37°C, but in man the capacity for vasoconstriction is poorly developed. This means that the heat produced internally is dissipated rapidly at the surface.

The internal temperature in most warm-blooded animals, including man, cannot be allowed to fall much below 37°C. Fane (1959) states that: "The average body temperature of swimmers returning to the ship was 34°C." However, he does not say how these temperatures were recorded and we doubt that this drop is representative of true internal temperature. The U. S. Navy Diving Manual (1963) states that: "A man can live and function effectively only if the temperature in his body remains close to normal: 98.6°F. (37°C)." We did not record internal temperatures, but presume these did not drop significantly during dives, a conclusion reinforced by the statements of Scholander, et.al., (1950a) that the least variable factor in homiothermy is body temperature, which is regulated to within a degree in most mammals, and of Spector (1956) that the body temperature of a naked man immersed to the neck in water of 6°C for 32-49 minutes dropped only to 36° from 37.5°C.

It would now be worthwhile to summarize pertinent data on the physiology of man at low temperatures and under basal conditions. The subject of hypothermia has been reviewed by The New York Academy of Sciences (1959). Excellent reviews of the problems involved in heat regulation and cold adaptation among animals are found in Scholander, et.al. (1950a & 1950b). It is well to point out that critical temperature (below which metabolic activity must be increased to maintain internal temperature) is directly related to surface area, metabolic rate and insulation and that these must be adjusted so that a balance is achieved between them and the temperature gradient between ambient and skin. This statement is basic to thermoregulation.

The critical temperatures for naked man are 26-27°C in air (Erikson, et.al., 1956) and 32-36°C in water (Fletcher, 1964). Lightly clothed man's critical temperature is about 14°C (Erikson, et.al., 1956). His energy requirement is in the vicinity of 3,000-3,200 Cal. per day for men of 25-45 years of age while "engaged in moderate physical activity" (Heinz Nutritional Data, 1958) or between 1.19 and 1.8 Cal./min. for supine or sitting man (Spector, 1956). Data differ somewhat on normal body skin temperatures maintained, but in general man maintains 30-34°C in room temperature air. The U. S. Navy Diving Manual (1963) states that the skin begins to feel cool when its temperature drops to 31, and cold at 30, when shivering may occur. These are probably body and not extremity temperatures, which would be lower. At a skin temperature of 15°C, the hands feel intense pain.

With these general data in mind the skin temperatures recorded on test dives become meaningful. It is obvious that motionless man cannot maintain normally high skin temperatures while wearing a neoprene exposure suit of 6 mm. thickness on arms and legs, 15 mm. (for three overlapping layers) on the torso, and 9 mm. (for two overlapping layers) on the head. This is to say that heat loss is not totally impeded, only slowed, in these thicknesses over a gradient of −1.9° for water to the necessary 30° for the skin. Nor should the exposure suit be thick enough to maintain such a gradient lest during diving activity the necessary dissipation of body heat be prevented unduly. Heat is dissipated from the surface to water totally by conduction, which is not the normal human method and therefore a careful balance must be achieved between temperature gradient, activity and suit thickness. A consideration of suit bulk, changing activity rates, the high conductivity of water and the relatively huge 30°C gradient will show that, at the present state of technology, man cannot hope to achieve thermal neutrality in polar waters, but is limited severely in exposure time, which we have shown to be in the vicinity of 30 minutes motionless at the surface, and estimate to be perhaps twice that long with moderate activity. These times are reinforced by similar statements of Neushul (1961), Fane (1959) and the U. S. Navy Diving Manual (1963).

Erikson, et.al. (1956) have said: "A warm-blooded animal usually first compensates for a falling environmental temperature by gradually increasing the insulation while the metabolism remains at resting level. At the critical temperature, insulation reaches its maximum and from there on heat balance is obtained by increasing heat production. This increase has been found to be rather closely proportional to the body-to-air temperature gradient, such as would be expected from Newton's Law of Cooling." As stated above, the partially inadequate insulation of the suit already places man in polar waters well below his critical temperature. Activity is the only heat-producing mechanism left and it is obvious that this has physiological limits. We have shown that heat production in terms of O_2 consumption is between 2.21 and 8.25 Cal./min. from the "resting" to "work" stages of a normal Antarctic sub-ice dive, which is from about twice that of a man, supine, at rest in air to that of a man walking at a fair pace up stairs (Spector, 1956). There was little terminal increase in metabolic rate on either of the test dives in which

O_2 consumption was measured, probably because we did not carry any test dive to this fairly extreme point. Shivering and the muscular activity of increased respiratory rate were the responses observed. When these occur, the diver should immediately ascend and emerge, as is stated by other papers on this subject, for what might be called "escape time" before severe cold stress sets in is short in polar waters, at times amounting to minutes only. However, barring the occurrence of cold stress, rewarming time is also short. Therefore we believe that repeated short dives are psysiologically more feasible than longer dives in which chilling is severe.

Two other methods of increasing diving time might also be mentioned. One is the consumption immediately before submergence of sugared water for the immediate caloric heat and glucose supply it gives. Another is a technological consideration. The polar diver breathes air at ambient temperature, which exposes his lungs and increases heat loss. Warming and insulation of diving tanks so that the diver continually breathes warm air might be of some aid in reducing heat loss, but the specific heat of air is small.

THE SUB-ICE ENVIRONMENT

Plates II and III show some features of the sub-ice environment but give little idea of the extreme clarity and good visibility of the waters at McMurdo Sound during October-November. The ice shown is a single season's accumulation of about 2.0-2.5 m. with a distinct ice crystal layer of up to 1.0 m. thickness on the under surface. From our experience plus valuable personal communication with Dr. Wohlschlag, Dr. Bunt and Mr. Verne Peckham, a picture emerges of water clarity as follows. At the winter's end, in September-October, the water and ice are more or less free of plankton and visibility is extremely good. It would be difficult to estimate the distance one could see, but on one occasion we had the opportunity to send a 30 m. line out horizontally from the diving hole and a Weddell Seal approximately 15 m. beyond the end of the line was clearly distinguishable. Dr. Wohlschlag (pers. comm.) reports photographing starfish and a fish trap clearly from the surface in 25 m. water depth. These distances approach the possible limits of clarity for water. During this "spring" period, various species of brown diatoms concentrate and "bloom" within the ice crystal layer. The diatoms do not interfere with clarity but do curtain out the available natural daylight so as to permit an estimated less than 1% penetration by late November and into December. Bunt (1964a and b) recorded 0.05-0.08% of surface illumination immediately below ice when the diatoms were at their peak. Concurrently, the water has been almost imperceptibly warming from –1.9° to about –1.7°C and this appears to have two effects. First, the under-ice crystal layer quite suddenly disappears, causing the diatoms to mix with the water. Second, the chrysophycean globular alga, *Phaeocystis*, is carried in by freshly introduced waters. Both of these factors serve to cloud the waters so that visibility may fall to 3 m. or less, but since the diatom curtain on the underside of the ice has largely disappeared, available light increases. Bunt (*op. cit.*) reported a light rise to 1.5% of available with the disappearance of the ice crystal layer, but states that it is difficult to separate light loss due to ice, diatoms and *Phaeocystis*.

When the ice goes out in mid to late summer, available light increases further and so does visibility. As fall and winter approach, available light decreases due to the declining position of the sun and the reduced photoperiod, but clarity increases further. Winter is once again dark but clear, which brings the season full cycle once again. There is a great deal of variation in every factor of this generalized story, but for diving practice one must separate light intensity and water clarity. Rarely does one have good conditions of both beneath ice in McMurdo Sound, at least not for photographic practice with any but fast black and white film. However, the naked eye is fully capable of adjusting to available light in under-ice surface waters (to 25 m. depth or a little more).

One peculiarity of Antarctic waters is shown in Plate III, Fig. 5. When water temperatures are still –1.9°C, the water contains crystals, some quite large, of ice which float freely. These are particularly noticeable in the beam of floodlighting equipment and presumably disappear when the water warms slightly in the summer.

MARINE LIFE

During the period in which we dived, McMurdo Sound was completely covered by heavy, solid sea ice and very little marine life was encountered. No bottom dives were made since the water under the diving hut was more than 50 fathoms deep. Weddell Seals were sighted on two occasions. The first time, a single adult male swam up into the ice hole while a diver was in the hole preparing for submergence. The seal ventilated there for 2-3 minutes (estimated) and backed out of the hole and departed. Two other divers were under the hole preparing for ascent when this occurred. The seal swam off about 50 m. just under the ice, turned around and returned. The divers continued their ascent and the seal swam to the last diver (Ray), took his left swim fin in its mouth, and rose into the ice hole with him and ventilated again for about two minutes. On another occasion, a seal swam to about 40 m., but appeared to be frightened by the floodlight array, the flashing Xenon light, or the divers, and did not venture closer. On no occasion was aggression evidenced.

Before diving in Antarctica, some consideration had been given to Leopard Seals, *Hydrurga leptonyx*, and Killer Whales, *Orcinus orca*, the most predatory of Antarctic animals. Neither was sighted. It was planned to monitor underwater sounds for the characteristic whoops of the Killer Whale as a safety measure, but since the nearest open water was several miles distant (about 20), this was not done.

No fishes were sighted in surface water, though species of *Trematomus* were being caught nearby by Biological Laboratory personnel. We looked among the ice crystal layer and suspect that fish might hide there, but could sight nothing. On one occasion a very large jellyfish was sighted from near the diving stage by Lavallee at a depth of about 25 m. The animal looked to be about 0.6 m. across the bell and was translucent blue-gray in color with long central tentacles, a dense peripheral array of short tentacles and was marked with radiating lines, about 6-8 in number.

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EXPLANATION OF THE PLATES

Plate I

Fig. 1. Diving hut on location on the sea ice of McMurdo Sound.

Fig. 2. Cutting through sea ice with chain saw.

Plate II

Fig. 3. Extracting sample of exhaled air from a diver just under the water surface. Polyethylene tubing is attached to the regulator exhaust. Note diving ladder construction.

Fig. 4. Looking up along the descending line to the diving stage and ice-hole.

Plate III

Fig. 5. Diver with light array of two 1,000-watt underwater floodlights, showing buoying and descending lines. Note reflections from ice crystals suspended in the water.

Fig. 6. Underside of the sea ice showing ice crystal layer. Darker areas are shadows and some diatom growth. Note close fit of mask around mouth and mask to minimize water-skin contact.

FIG. 1

FIG. 2

SELF-CONTAINED DIVING OPERATIONS IN McMURDO SOUND, ANTARCTICA: OBSERVATIONS OF THE SUB-ICE ENVIRONMENT OF THE WEDDELL SEAL, LEPTONYCHOTES WEDDELLI (LESSON)

FIG. 3

FIG. 4

SELF-CONTAINED DIVING OPERATIONS IN McMURDO SOUND, ANTARCTICA: OBSERVATIONS OF THE SUB-ICE ENVIRONMENT OF THE WEDDELL SEAL, LEPTONYCHOTES WEDDELLI (LESSON)

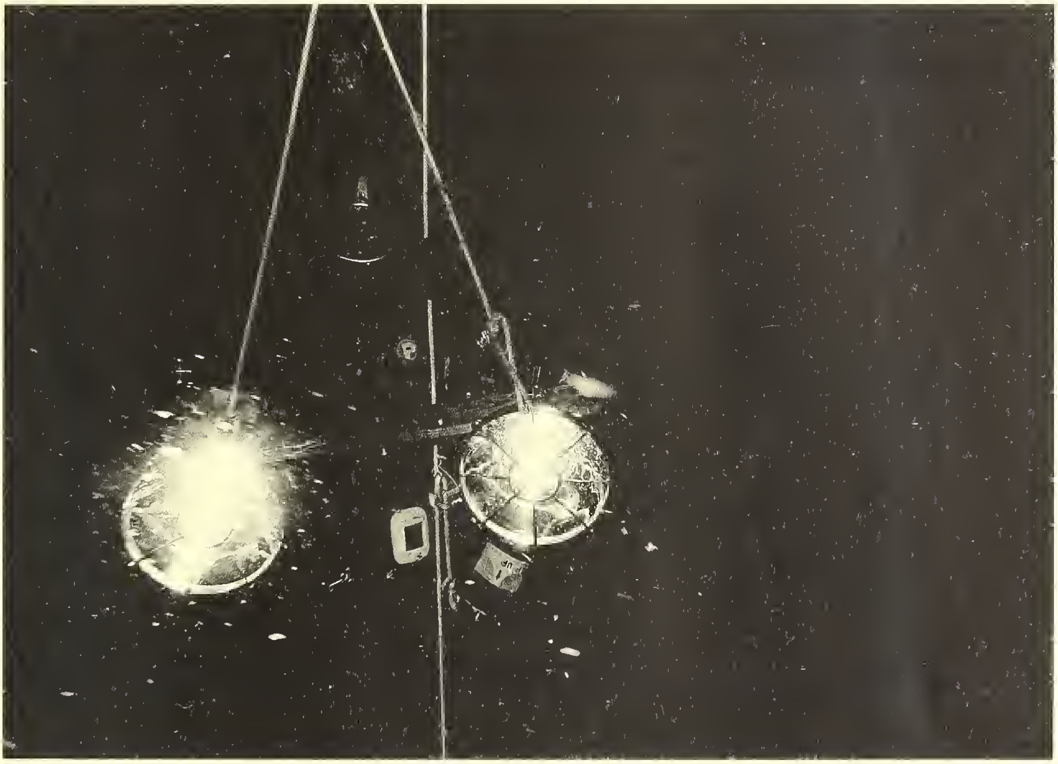

FIG. 5

FIG. 6

SELF-CONTAINED DIVING OPERATIONS IN McMURDO SOUND, ANTARCTICA: OBSERVATIONS OF THE SUB-ICE ENVIRONMENT OF THE WEDDELL SEAL, LEPTONYCHOTES WEDDELLI (LESSON)

9

Birds, Butterflies, and Plant Poisons: A Study in Ecological Chemistry[1]

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[This paper is a contribution from the William Beebe Tropical Research Station of the New York Zoological Society at Simla, Arima Valley, Trinidad, West Indies. The Station was founded in 1950 by the Zoological Society's Department of Tropical Research, under Dr. Beebe's direction. It comprises 200 acres in the middle of the Northern Range, which includes large stretches of government forest reserves. The altitude of the research area is 500 to 1,800 feet, with an annual rainfall of more than 100 inches.

[For further ecological details of meteorology and biotic zones see "Introduction to the Ecology of the Arima Valley, Trinidad, B.W.I.," by William Beebe, Zoologica, 1952, Vol. 37, No. 13, pp. 157-184].

I. INTRODUCTION

IN 1862, Bates proposed the theory of mimicry which included the idea that certain sexually mature insects are brightly colored and behave conspicuously in order to advertise to vertebrate predators the fact that they are unpalatable. Wallace (1867a) extended this to include gaudily colored caterpillars and the experiments of Weir (1869, 1870) and Butler (1869) led to the establishment of the theory of warning coloration (Wallace, 1869, 1870; Darwin, 1871; Poulton, 1887). Slater (1877) first suggested that the unpalatability of warningly colored insects is derived from their larval foodplants, stating that "strikingly colored insects, not otherwise specially protected, will be found to feed upon poisonous plants or upon such as, though not poisonous, possess unpleasant, or at least very powerful odours or flavours" (p. 205). This was accepted by Meldola (1877), and considered probable but not exclusive by Wallace (1889). Seven years later, Hasse (1896) produced his monumental study of mimicry in butterflies which, along with the works already mentioned, firmly established the hypotheses that (1) species of butterflies differ in their palatability to vertebrate predators, (2) the palatability is correlated with their taxonomic groupings, and (3) those groups which are distasteful feed as larvae upon poisonous plants.

According to the early findings, there are five major groups of unpalatable butterflies. These are (see also Table 1): (1) the tribe Troidini of the subfamily Papilioninae, known as the "pharmacophagus" swallowtails which feed on *Aristolochia* and related plants of the family Aristolochiaceae (Asaraceae). Both the butterflies and the plants are abundantly represented in the New and Old World tropics (excluding Africa) and a few of each extend into temperate regions. (The geographic distribution of the butterflies is based on Seitz, 1924, and that of the plants on Good, 1947). Within the large family Nymphalidae there are four distasteful taxa. Two closely related subfamilies, (2) the Ithomiinae and (3) the Danainae, are both reputed to be unpalatable. The Ithomiinae are almost exclusively neotropical and feed mainly on the Solanaceae. These plants have a distribution similar to that of the Aristolochiaceae. The Danainae are pantropical, but best developed in Africa and the Indo-Australian region, and feed predominantly upon Asclepiadaceae and the related Apocyanaceae, both of which are pantropical, with one genus, *Asclepias*, well developed in North America. The remaining two subfamilies of distasteful species are (4) the Acraeinae and (5) the Heliconiinae. Both feed upon Passifloraceae, another pantropical plant family, but the Acraeinae have been reported from other plants as well (Hasse, 1896; Fawcett, 1901, 1903;

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Wynter-Blyth, 1957). The heliconiines are exclusively neotropical with a few species reaching the subtropics, while the acraeines are found predominantly in the Old World tropics.

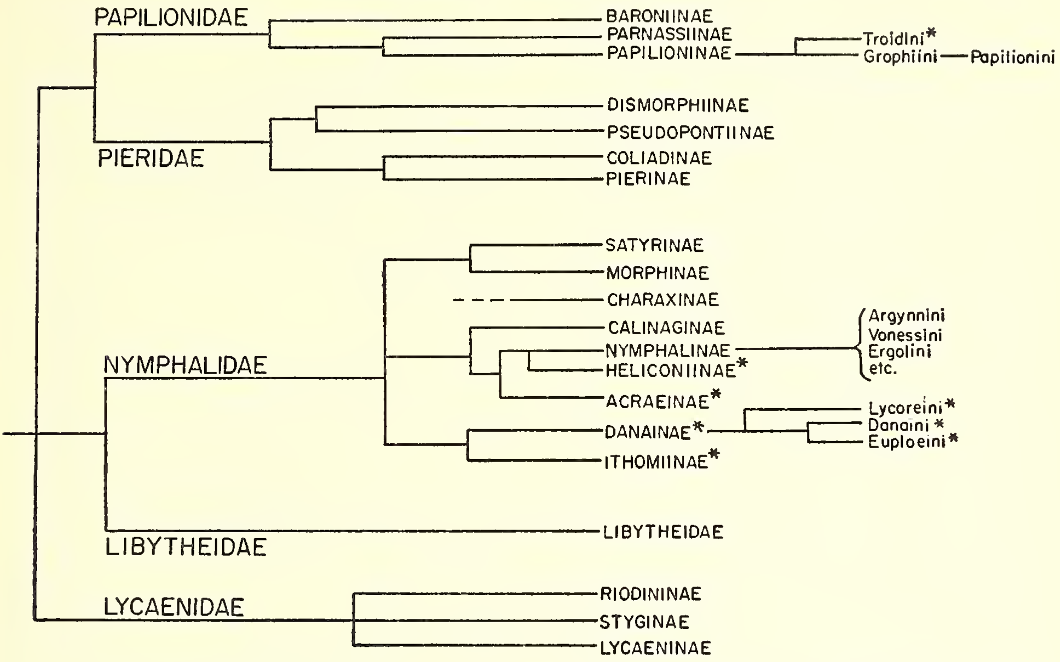
The Pieridae were not studied in the experiments presented in this paper, but it appears that some are palatable or Batesian mimics, others are unpalatable and serve either as models or enter into Müllerian mimicry complexes, while still others seem of intermediate acceptability. Thus Wallace (1867b) believed the Indo-Australian pierine genus *Delias (Thyca)* to be unpalatable due to the coloration and conspicuous behavior of the butterflies and because certain of the species appear to be mimicked by other, presumably palatable, pierines. Finn (1895, 1897a and b) experimentally demonstrated that *Delias eucharis* Drury was unacceptable to a variety of vertebrate predators. Similarly, the African genus *Mylothris* is also unpalatable, as Swynnerton (1919) established. The neotropical genus *Pereute,* which is very closely allied to *Delias,* (Klots, 1933), is probably also unpalatable and the species may be Müllerian mimics of some of the heliconiines, as Dixey (1894) first suggested. Similarly, the fascinating South American genus *Archonias (Euterpe),* now revised to include the former genus *Charonias,* (Klots, 1933), seems to enter a Müllerian complex with both the *Aristolochia* swallowtails and the "tiger-stripe" complex of yellow, black and orange ithomiines, heliconiines and lycoreines. One wonders if Bates' classical *Leptalis (Dismorphia)* species upon which his theory of mimicry was based are also unpalatable, as Beddard (1892) suggested. However, the fact that they belong to the most primitive subfamily, Dismorphiinae, makes this unlikely for the reasons discussed in Section III-D-5. Further indication of the extreme variability in this family is seen even within a single species: for example, the larvae of *Pieris brassicae* Linnaeus appear unpalatable, whereas the adults are relatively acceptable (Weismann, 1882; Pocock, 1911). On these lines of evidence, and also on the basis of the feeding experiments of Marshall (1902), Carpenter (1921), Finn (1896) and Frazer & Rothschild (1960), it seems best to state provisionally that the Pieridae are intermediate between the five unpalatable groups enumerated above and the palatable Papilionini, Satyrinae, Nymphalinae and Lycaenidae.

Too little is known of the remaining groups in Table 1 to make valid statements concerning their relative acceptability. However, on the basis of both coloration and behavior, it is likely that the Parnassiinae are unpalatable, while the Morphiinae and Libytheidae are palatable. Finally, it is well to remember that exceptions to this general picture will undoubtedly be found. Thus, for example, the nymphaline genus *Adelpha* and the riodinine genus *Nymphidium* have species which mimic each other in the neotropics (Seitz, 1924) and yet both belong to groups considered palatable. Another peculiar nymphaline genus is *Agrias.* These butterflies are among the most gaudily colored members of the animal kingdom and possess complex scent organs. Both of these facts argue that they should be an unpalatable group involved in Müllerian mimicry (L. Brower, 1963), and yet their behavior, typical of cryptic species (Seitz, 1924), supports the classical contention that they are palatable.

While these considerations are no doubt generally correct, it must be emphasized that nearly all the evidence has been obtained from anecdotal accounts or from experiments conducted without adequate controls. As a result, it has not been possible to make quantitative statements regarding the comparative palatability of the numerous species studied. Yet this is a situation greatly to be desired both to provide a solid groundwork for biochemical investigation of the unpalatability of insects in relation to the plants which they eat, and to gain a basis for understanding the evolutionary relationships between the different groups of butterflies as seen in mimicry.

It is towards these ends that the present paper is aimed. In it we shall (1) describe the results of an experiment which quantitatively demonstrates that the groups of butterflies classically held to be unpalatable are in fact so. Following this there will be (2) a discussion of the evolution of unpalatability and the bearing of the experimental findings on Batesian and Müllerian mimicry. Then, (3) by a survey of the phytochemical literature, we shall establish that the groups of plants which are eaten by the larvae of the unpalatable butterflies contain extremely poisonous substances. Finally, we shall attempt to support the thesis that (4) phytophagous insects in the course of their evolution have developed the ability to incorporate poisonous and repellent plant chemicals into their tissues with the result that they are unpalatable to vertebrate predators. The paper will not discuss how a mutation allowing unpalatability to develop can be of advantage to an individual organism prior to its establishment in the population. This genetical problem was originally considered by Eltringham (1909), and Fisher (1958) suggested a plausible solution which Huheey (1961) has recently elaborated.

TABLE 1. PROBABLE RELATIONSHIPS OF THE FAMILIES, SUBFAMILIES AND SOME TRIBES OF THE BUTTERFLIES. BASIC SCHEME MODIFIED FROM EHRLICH (1958). SEE ALSO REFERENCES IN TABLE 2. * = CLASSICAL UNPALATABLE GROUPS.



II. ACKNOWLEDGMENTS

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III. THE EXPERIMENT

(A). *Materials and Methods*

From October through December, 1962, six Blue Jays, *Cyanocitta cristata bromia* Oberholser, were trapped in mist nets at Amherst, Massachusetts, and maintained in an aviary for several weeks. They were then transported in a cage to Trinidad, W. I., as hand luggage over a period of 30 hours by air and car. The cage consisted of six individual units 8 inches high by 6 inches wide by 12 inches long, each with a wire screening front, a perch, and with food and water. Upon arrival, the birds were placed in an outdoor aviary in individual experimental cages similar to those at Amherst and described in detail elsewhere (Brower, Brower & Collins, 1963).

From January, 1963, until the experiments were completed, the birds were fed a standard diet of broiler chicken mash mixed with crushed yolks of hard boiled eggs, fine-chopped liver, heart or beef, crushed banana and a small amount of water. This was supplemented by sunflower seeds and paw-paw. A tube of water was kept in each cage and the sand-covered floor was kept clean by periodic sifting. The sex of the jays was not determined.

The ten species of butterflies studied are listed in Table 2, along with their known or suspected larval foodplants. In order to eliminate the variables associated with the two sexes, which include the color-pattern, odor and scent organs, only one sex was offered to the birds. The male was chosen, since females are less easily obtained in the wild. All the butterflies were caught in various parts of Trinidad during the summer of 1963, and only specimens in good condition were offered to the birds. The species were intact, except *Lycorea ceres* from which the scent-disseminating hairpencils had been removed for other purposes. As F. Müller (1878) originally suggested for the lycoreine genus *Ituna*, it is possible that these hairpencils are defensive as well as sexual organs, because they are extruded when the male is held. This is apparently also true of the Euploeini (Varley, pers. comm.), but is not true of *Danaus* (Brower & Cranston, in press). Whether the hairpencils of *Lycorea* enhance its unpalatability is not yet known, and the conclusions in this paper are made on the assumption that they do not.

All species were killed by freezing, spread horizontally, and stored in this position in glassine envelopes (Ward's Natural Science Establishment, Rochester, New York) for two days to two weeks in a deep-freezer (see Brower, Brower & Collins, 1963, for details of preparation). The butterflies were thawed a few minutes before use. This freezing technique greatly facilitates the experimental work, but one possible drawback, as mentioned in our previous paper, is that acetylcholine is inactivated. Fowler & Lewis (1958) have shown that this is due to the action of cholinesterase, which is released when the tissue thaws. Acetylcholine is one of the pharmacologically active substances frequently associated with venoms, venomous tissues or stinging organs in both plants and animals, and probably enhances the effects of the poisons on the victims or increases the responsiveness of their tissues to the noxious substances (Frazer & Rothschild, 1960; Bisset, Frazer, Rothschild & Schachter, 1960). Whether it plays any role in the unpalatability of the butterflies studied is not yet known, but the possibility should be kept in mind.

TABLE 2. CLASSIFICATION OF THE 10 SPECIES OF BUTTERFLIES OFFERED TO THE BLUE JAYS, AND THEIR FOODPLANTS*

BUTTERFLIES				FOODPLANTS	
Species[1]	Family	Subfamily	Tribe	Species	Family
Parides neophilus parianus (Hübner)	Papilionidae	Papilioninae	Troidini[2]	*Aristolochia* spp.[7]	Aristolochiaceae
Euptychia spp.	Nymphalidae	Satyrinae	Satyrini[3, 4]	Various species? [5, 11]	Graminae
Heliconius sara thamar Hübner	Nymphalidae	Heliconiinae	Heliconiini[4, 5]	*Passiflora auriculata*[8]	Passifloraceae
Agraulis vanillae vanillae (L.)	Nymphalidae	Heliconiinae	Heliconiini[4, 5]	*Passiflora foetida*[8]	Passifloraceae
Biblis hyperia (Cramer)	Nymphalidae	Nymphalinae	Ergolini[5]	*Tragia volubilis*[9, 11]	Euphorbiaceae
Anartia amalthea amalthea (L.)	Nymphalidae	Nymphalinae	Vanessini[5]	Various species? [5, 11]	Acanthaceae
Victorina steneles steneles (L.)	Nymphalidae	Nymphalinae	Vanessini[5]	*Blechum brownei*[9, 12]	Acanthaceae
Danaus plexippus megalippe (Hübner)	Nymphalidae	Danainae	Danaini[6]	*Asclepias curassavica*[7, 11]	Asclepiadaceae
Lycorea ceres ceres (Cramer)	Nymphalidae	Danainae	Lycoreini[6]	*Gonolobus broadwayi*[7]	Asclepiadaceae
Ithomia drymo pellucida Weymer	Nymphalidae	Ithomiinae	Ithomiini[4]	Various species?[10]	Solanaceae

*The bases for this classification and the larval foodplant records are:

[1] All subspecific names from Crane (1954) except *H. sara thamar*, which is from Alexander (1961); [2] Munroe (1960) and Munroe & Ehrlich (1960); [3] Clark & Clark (1951); [4] Ehrlich (1958); [5] Seitz (1924); [6] Forbes (1939); [7] present paper, see text; [8] Alexander (1961); [9] Kaye (1921); [10] probable, from [9] and [11]; [11] W. Müller (1886); [12] W. Müller (1886) gives *Stephanophysum longifolium* and other Acanthaceae for the Brazilian *Victorina trayga* Hbn.

The experiment was conducted between 8 a.m. and 12 noon for ten days from August 6 to 15, 1963, inclusive. Food was removed from the birds' cages at 8 a.m. and after about two hours of deprivation they were given the butterflies. In order to place the butterflies in each bird cage without excessively disturbing the occupant, the tray floor was pulled out 12 inches and they were placed dorsal side up on the sand with 12-inch forceps. Because the movements of the birds tended to disturb the arrangement of the butterflies, a pinch of sand was placed on the apices of the fore and hind wings of each. This was done without obscuring their color-patterns. Each tray was then pushed back into position. After a two-hour period, the trays were pulled out and the condition and position of the ten butterflies were noted in detail. All remains of wings and bodies and all intact individuals were removed and tabulated for scoring purposes as in the earlier experiments (not touched, pecked, killed or eaten, J. Brower, 1958a). To avoid confusion it should be emphasized that killed means that a bird mutilated the frozen, dead butterfly to an extent that, had it been alive, it would have died or been unable to reproduce. A dish of fresh food was returned to each cage immediately after the removal of the butterflies at the end of a day's experiment.

(B). *Experimental Design*

This experiment was designed to compare the palatability of ten species of butterflies to six birds as experimental predators. Five of these butterflies were from groups reputed to be unpalatable, whereas the other five were from groups assumed to be edible. At 24-hour intervals, each bird was presented with an array of 10 butterflies, one each of the 10 species, and was allowed two hours to respond to them. This was repeated for 10 consecutive days so that each bird received a total of 100 butterflies. The positions of the butterflies with respect to each other were the same for all birds on any one day, but were varied at random from day to day. This was done as follows: the butterflies were placed on the floor of each cage in three rows, one with four individuals, and two with three. All butterflies were about one inch apart. Each species was assigned a permanent number from 1 to 10. Slips of paper with these numbers were drawn at random. The butterfly represented by the first number to be drawn was placed in the back row on the left, the second in the same row to the right of the first, and so on, until four had been set out. Then three more were placed in a middle row, and the remaining three in the front row, the last being placed in the right-hand position. In this way, a trapezoid of approximately equidistant butterflies was set out, with its base towards the back of each cage.

A criterion for qualification of the predators used in all of our mimicry experiments has been that they must eat at least one of the insect species offered. On this basis, one Blue Jay in this experiment was disqualified because it failed to eat any of the 10 species of butterflies (Table 3).

It is important to emphasize that the birds were caught as adults in North America and shipped to the neotropics; this assured that they could never have had prior experience with any of the butterflies. Moreover, with the exception of the satyrines, *Euptychia* spp., and the danaine, *Danaus plexippus megalippe,* all of the butterflies were of a radically different color-pattern from anything the birds could have seen previously. This is an important difference between this investigation and our earlier experimental studies of mimicry where prior experience with the insects was either indicated or at least a possibility. Another way of obtaining naive predators would have been to hand-rear native birds under a carefully controlled food regimen. But this is costly in time and effort, and was not feasible. Moreover, hand-reared birds may become juvenilized, that is, excessively dependent upon the experimenter, the feeding apparatus, or even the example of another bird, in getting food, and thereby may require an abnormally long time to learn to reject items found to be unpalatable (Swynnerton, 1915, *in* Carpenter, 1942; Rand, 1942).

There are several other differences from the earlier work. The butterflies were presented simultaneously instead of sequentially, one at a time. Previously, insects of only two appearances were offered to the predators: that of the edible, and that of the model and mimic. In this experiment, the 10 species all differed from each other in size, shape and color-pattern (see black and white illustrations in Crane, 1954; colored illustrations in Seitz, 1924). Again in the earlier work, the experimental and control predators had only two possible differences of palatability to cope with during the learning period; here, there were ten possibilities. Instead of repeated presentations of short duration (1½–2 minutes), the jays in this experiment were allowed two hours to examine and respond to the butterflies. Finally, the total number of presentations of each butterfly species was 10 in this experiment, while in the earlier ones it was generally more. The main consequence of these differences in design is that the birds in the present experiment could not be expected to learn to avoid the butterflies on sight alone. The number of the three most unpalatable species which the

TABLE 3. REACTIONS OF 6 SINGLY-CAGED BLUE JAYS TO 10 SPECIES OF NEOTROPICAL BUTTERFLIES
(NT=not touched; P=pecked; K=killed; E=eaten)

	BUTTERFLIES																																												
	Parides				*Danaus*				*Ithomia*				*Heliconius sara*				*Lycorea*				*Biblis*				*Euptychia*				*Agraulis*				*Anartia*				*Victorina*				Totals				
Bird No.	NT	P	K	E	NT	P	K	E	NT	P	K	E	NT	P	K	E	NT	P	K	E	NT	P	K	E	NT	P	K	E	NT	P	K	E	NT	P	K	E	NT	P	K	E	NT	P	K	E	Total
1	3	6	1	0	6	4	0	0	5	5	0	0	5	5	0	0	9	1	0	0	5	5	0	0	5	5	0	0	8	2	0	0	3	7	0	0	5	5	0	0	54	45	1	0	100
2	0	7	3	0	1	4	4	1	0	4	2	4	0	2	3	5	0	0	1	9	0	1	2	7	0	0	0	10	0	0	0	10	0	0	1	9	0	0	2	8	1	18	18	63	100
3	0	2	8	0	0	2	8	0	1	1	6	2	0	0	4	6	0	1	6	3	0	0	1	9	0	0	1	9	0	0	0	10	0	0	0	10	0	0	0	10	1	6	34	59	100
4	3	6	1	0	3	4	3	0	4	4	1	1	2	3	4	1	2	0	5	3	1	0	1	8	0	0	0	10	0	0	2	8	0	0	1	9	1	0	0	9	16	17	18	49	100
5	1	3	6	0	0	0	10	0	1	2	4	3	1	1	2	6	0	0	6	4	0	0	1	9	1	0	0	9	0	0	3	7	0	0	0	10	0	0	0	10	4	6	32	58	100
6	3	5	2	0	2	2	6	0	3	6	1	0	1	5	3	1	0	0	0	10	2	0	1	7	3	1	0	6	0	0	1	9	1	0	2	7	0	0	1	9	15	19	17	49	100
Totals+	7	23	20	0	6	12	31	1	9	17	14	10	4	11	16	19	2	1	18	29	3	1	6	40	4	1	1	44	0	0	6	44	1	0	4	45	1	0	3	46	37	66	119	278	500

+Bird No. 1 is not included in the totals because it failed to eat any butterflies (see text).

five birds avoided on sight alone (not touched) was 22/150 (15%), whereas the number which these same five killed was 65/150 (43%) (data in Table 3). These results can be compared with those from an earlier experiment in Florida (J. Brower, 1958b) in which three Scrub Jays, *Aphelocoma coerulescens coerulescens* (Bosc), were offered the unpalatable *Battus philenor* (Linnaeus) and one edible butterfly species. The combined results for the first ten presentations showed 14/30 (47%) of the *B. philenor* not touched, and 10/30 (33%) killed. Another unpalatable species, *Danaus plexippus plexippus* (Linnaeus), tested in the same way with these three birds plus one other, resulted in 11/40 (28%) not touched, and 9/40 (23%) killed. In other words, the birds in the present experiment killed far more than they rejected on sight, while in the two experiments just described, as well as in another one (Brower, Brower & Collins, 1963), the reverse was true. It is therefore clear that learning was substantial in the earlier experiments, but negligible in the present one. Here the birds apparently attacked most of the butterflies each day and went on to eat only those which were palatable. For this reason the measure of palatability in this investigation will be based on the number of butterflies not eaten (= not touched, pecked or killed) *vs.* eaten.

(C). *Results and Statistical Analysis*

The reactions for all six birds are given in Table 3 where the ten species of butterflies are arranged in order of increasing acceptability from left to right. For the reasons stated above, Bird No. 1 is not included in the totals. Table 4 shows the individual and mean relative frequencies and variances of the butterflies not eaten by the five birds. The differences which are apparent among the species in Table 4 are analyzed in Table 5. Dixon & Massey (1957) and Snedecor (1946) were consulted for statistical procedures and the .05 level of formal significance was chosen. The analysis is similar to that of our earlier study (Brower, Brower & Collins, 1963) with one refinement: the data were transformed (see Dixon & Massey, pp. 182-183; Snedecor, p. 449). This was done because the data were tabulated in the form of proportions (*i.e.*, the frequency not eaten, p, *vs.* pecked, killed, or eaten, q, in which p + q = 1). The transformation results in a closer approximation of the individual frequencies to the normal distribution, thereby making the variances independent of the proportions and so providing a more accurate basis for carrying out the analysis of variance. It would have been possible to do the analysis on the actual numbers not eaten because the number of butterflies given to

Table 4. Relative Frequencies, Means, Variances and Transformed Variances* of 10 Species of Neotropical Butterflies Not Eaten by 5 Blue Jays
(Data from Table 3; for statistical analysis, see Table 5)

	BUTTERFLIES									
Bird No.	*Parides*	*Danaus*	*Ithomia*	*Heliconius sara*	*Lycorea*	*Biblis*	*Euptychia*	*Agraulis*	*Anartia*	*Victorina*
2	1.00	.90	.60	.50	.10	.30	.00	.00	.10	.20
3	1.00	1.00	.80	.40	.70	.10	.10	.00	.00	.00
4	1.00	1.00	.90	.90	.70	.20	.00	.20	.10	.10
5	1.00	1.00	.70	.40	.60	.10	.10	.30	.00	.00
6	1.00	1.00	1.00	.90	.00	.30	.40	.10	.30	.10
Means	1.00	.98	.80	.62	.42	.20	.12	.12	.10	.08
Variances	.000	.003	.025	.068	.118	.010	.027	.017	.015	.007
Transformed Variances*	.00	66.3	230.8	282.5	671.3	54.0	265.3	231.3	200.0	145.5
	Unpalatable			Intermediate		Palatable				

*See text.

the birds was the same for all species. However, in a study of this kind, it is not always possible to obtain the data exactly as planned; if one bird had died on the ninth day, it would have necessitated converting the data to proportions. Some of the predators died or stopped eating in our previous investigation (Brower, Brower & Collins, 1963) and probably will in future ones. Consequently, for consistency with our past and anticipated work, as well as to facilitate direct comparison, the analysis seemed best carried out on the data in the form of proportions.

1. Relative Palatability of the Ten Species

An examination of Table 4 shows that *Parides* was uniformly not eaten, while all the other species were variably treated. Bartlett's test for the homogeneity of the 10 variances shows lack thereof ($P < .001$, Table 5a). When *Parides* is excluded, and the remaining nine are compared, there is no significant difference in their variances as a group ($.50 > P > .25$, Table 5a). It is thus necessary to consider *Parides* separately from the rest of the species in the statistical analysis.

The F test applied to the remaining nine species indicates that the differences of their means are highly significant ($P < .001$, Table 5b). The components of variance analyses shown in this table indicate that the differences result from the butterflies being different (Column category) rather than the birds differing among themselves in their behavior towards the butterflies (Row category, $.25 > P > .10$). In order to make simultaneous comparisons between all the means by pairs, the q statistic was used with 95% confidence limits (Dixon & Massey, pp. 153-155). This analysis is shown in Table 5c where "yes" indicates that the paired means are significantly different, and "no" that they are not.

As shown above, *Parides* is completely unpalatable to the Blue Jays. *Danaus* and *Ithomia* are only slightly less so. *Heliconius sara* and *Lycorea* occupy an intermediate position between these species and the remaining five, *Biblis, Euptychia, Agraulis, Anartia* and *Victorina*. These latter five can be considered relatively palatable. The differences between the members of the unpalatable and palatable groups are all significant, but the differences within these two groups are not. The two intermediate species do not differ significantly from each other. However, *Lycorea* is more palatable than *Danaus*, although not significantly less acceptable than the five palatable species. This lack of significance results from the large variance in the treatment of *Lycorea*. *Heliconius sara* is significantly more palatable than *Danaus*, and significantly less palatable than four of the five palatable species, *i.e.*, all except *Biblis*. Thus the breaks apparent in Table 4 are real, with the first three species not eaten 80% or more of the time, the last five eaten 80% or more of the time, while *Heliconius sara* and *Lycorea* were eaten about half the time. These results substantiate the classical statements in the literature to a remarkable extent.

2. The Species of Intermediate Palatability

Further examination of Table 4 indicates that the two species which are of intermediate palatability, *Lycorea* and *Heliconius sara*, have the greatest variances. This resulted from the large range in the frequencies not eaten by the five individual birds. These were .70 to .00 for *Lycorea* and .90 to .40 for *Heliconius sara*. While Bartlett's test has shown that the variances of the nine species as a group do not differ significantly, paired comparisons indicate that the variance of *Lycorea* is significantly higher than

TABLE 5. STATISTICAL ANALYSES OF THE DATA IN TABLE 4*

a. Homogeneity of the variances (Bartlett's test).

1. All the species:
   F=3.23 d.f.=9/1375 P<.001

2. Nine species (excluding *Parides neophilus*):
   F=1.01 d.f.=8/1163 .50>P>.25

b. Variance analysis of the nine means, excluding *Parides neophilus* (F test).

Variance categories	Sum of squares	Degrees of freedom	Mean square	F ratio	P
Birds (Rows)	1541	4	385	1.75	.25>P>.10
Butterflies (Columns)	28635	8	3579	16.27	<.001
Butterflies × Birds (Residual)	7046	32	220	—	—
TOTALS	37222	44	—	—	—

c. 95% confidence limits of the nine means by pairs, excluding *Parides neophilus* (q test).

	Victorina	*Anartia*	*Agraulis*	*Euptychia*	*Biblis*	*Lycorea*	*Heliconius*	*Ithomia*
Danaus	YES	YES	YES	YES	YES	YES	YES	NO
Ithomia	YES	YES	YES	YES	YES	NO	NO	
Heliconius	YES	YES	YES	YES	NO	NO		
Lycorea	NO	NO	NO	NO	NO			
Biblis	NO	NO	NO	NO				
Euptychia	NO	NO	NO					
Agraulis	NO	NO						
Anartia	NO							

"YES" indicates that the means are significantly different, "NO" that they are not

d. Variance analyses of the variances by pairs, excluding *Parides neophilus* (F test).

1. *Lycorea ceres vs. Biblis hyperia:*
   F=12.43 d.f.=4/4 .05>P>.025
2. *Lycorea ceres vs. Danaus plexippus megalippe:*
   F=10.13 d.f.=4/4 .05>P>.025
3. *Lycorea ceres vs. Victorina steneles:*
   F=4.61 d.f.=4/4 .20>P>.10
4. All remaining paired comparisons:
   Not significant by inspection.

*This analysis was performed on the data in Table 4 transformed according to the method of Snedecor, see text.

both that of *Danaus* in the unpalatable group and *Biblis* in the palatable group (Table 5d). Thus species that are either highly palatable or very unpalatable were treated uniformly. In contrast, the species of intermediate palatability were treated variably. The biological implications of this distinction are of great interest and will be discussed below (Section III-D-4).

(D). *Discussion*

1. Palatability Differences among the Species

The results of the experiment indicate that the ten species of butterflies fall into three groups ranging from acceptable to completely unacceptable to the five Blue Jays (Table 4). The anticipated outcome was obtained for *Parides* and *Danaus,* close relatives of which had already been shown to be unpalatable to another species of jay (J. Brower, 1958a, b). *Ithomia* was also found to be unacceptable, confirming for one species the classical prediction that members of this subfamily would be distasteful. *Heliconius sara,* already demonstrated to be unpalatable to Silverbeak Tanagers (Brower, Brower & Collins, 1963), was also rejected more than half of the time by the Blue Jays. The finding that

Lycorea is intermediate was unexpected, because we anticipated that it would be closer to *Danaus* than it proved to be. The remaining five species were all relatively palatable to the Blue Jays.

2. Phylogenetic Correlations

For the theoretical reasons discussed by Brower, Brower & Collins (1963) (see also Section III-D-5), it was predicted that palatability, like morphology, would prove to be a more conservative taxonomic character in the Lepidoptera than color-pattern. On the basis of comparative morphology, it has been maintained that *Agraulis vanillae* is close to the nymphalid stock from which the Heliconiinae are derived. Because of this, the palatability of *A. vanillae* was predicted to be higher than that of the other heliconiines studied and in fact was shown to be (Brower, Brower & Collins, 1963). This has now been independently confirmed with Blue Jays which ate *A. vanillae* to a greater extent than *H. sara.* Similar confirmation of the accepted taxonomic scheme has been shown for *Lycorea* and *Danaus,* which belong to two tribes of the Danainae (Table 1). Following the classification of Forbes (1939), *Lycorea* is nearer to the ancestral nymphalid stock than *Danaus,* and the finding that *Lycorea* is the more palatable supports this. Another related pair, *Anartia* and *Victorina,* are in the tribe Vanessidi of the subfamily Nymphalinae. Although radically different in color-pattern, these two species are placed in adjacent genera by Seitz (1924) and were regarded as very closely related by W. Müller (1886). The fact that both are highly acceptable to the Blue Jays agrees with this. Finally, these two species, together with *Biblis,* support the hypothesis that the Nymphalinae in general are palatable.

Table 6 summarizes the comparative palatability of all the species which have been investigated to date in controlled, quantitative experiments. From this it can be seen that the present study, along with the previous ones, confirms the classical notions regarding the palatable and unpalatable groups of butterflies. Moreover, they establish that palatability is a valuable taxonomic character which ranks with morphology in determining phylogenetic relationships.

3. Potential Batesian Mimics

The results obtained also bear upon possible mimetic relationships between certain neotropical butterflies. On the basis of the finding that *Victorina s. steneles* is highly palatable, it can now be stated that it is a potential Batesian mimic of the heliconiine species *Philaethria dido dido* Clerck with which it is sympatric. *Philaethria* is bright green with black and brown markings. *Victorina* shares this color-pattern on the upper surface of its wings but, like many nymphalines, is cryptic on the undersurface. Thus if *Philaethria* proves to be distasteful, *Victorina* is a doubly protected species (Ford, 1955): by mimicry when flying, but by inconspicuousness when at rest.

In view of the fact that the nymphalines, *Biblis* and *Anartia,* are both palatable, their conspicuous coloration requires explanation because there are no known distasteful species which they resemble in detail. The alternative that their coloration functions solely as social releasers is unlikely since *Biblis* is a monotypic genus in Trinidad and apparently throughout its range, and *Anartia* includes only one other species in Trinidad besides *A. amalthea,* which is said to occupy a separate habitat (Kaye, 1921; Seitz, 1924). *Biblis* has a striking crimson border on the outer margin of black *hindwings* and solid black forewings. The upper surface of both sets of wings in *Anartia* bears a less discrete pattern of blackish-brown borders, white spots and single vertical red bands. The under surface of *Biblis* is similar to the upper surface, whereas that of *Anartia* is cryptic, being tan and mottled. The coloration of *Biblis,* and *Anartia* to a lesser extent, recalls in a general way that of the highly unpalatable Müllerian mimics, *Heliconius erato hydara* Hewitson and *Heliconius melpomene euryades* Riffarth. Both of these are velvet black with crimson patches on the *forewings.* There seems to be justification for the suggestion that *Biblis* and *Anartia* may derive some advantage from Batesian mimicry of these two *Heliconius.* This is based on the finding (Brower, Brower & Collins, 1963; J. Brower, 1963) that birds can generalize from experience with an unpalatable butterfly to reject on sight another which is broadly similar in size and shape, but totally unlike it in color-pattern. These two nymphalines could profitably be used in experiments to estimate the relative Batesian advantages of differing degrees of crude resemblance to a model such as *H. erato* or *H. melpomene.*

4. The Variable Treatment of Species of Intermediate Palatability and Its Bearing on the Dynamic Relationship of Batesian and Müllerian Mimicry

From an evolutionary point of view, a very interesting aspect of this study concerns the species of intermediate palatability which were treated in an extremely variable manner by the birds. A similar situation occurred in the earlier study (Brower, Brower & Collins, 1963), where the variances in treatment of the three least unpalatable heliconiines were seven or more times greater than those of the four very unpalatable

Table 6. Summary of Comparative Palatability of Butterflies in Controlled Experiments with Singly-caged Avian Predators

Species	Taxonomic Tribe	Predator	Per Cent Not Eaten Palatable ⟶ Unpalatable					Reference
			≤10	11-29	30-70	71-89	≥90	
Papilio troilus L.	Papilionini	Scrub Jay	X					J. Brower, 1958 b, c.
Papilio palamedes (Drury)	Papilionini	Scrub Jay	X					J. Brower, 1958 a, c.
Papilio glaucus L.	Papilionini	Scrub Jay	X					J. Brower, 1958 a, b, c.
Papilio polyxenes (Fabr.)	Papilionini	Scrub Jay	X					J. Brower, 1958 b.
Battus philenor L.	Troidini	Scrub Jay					X	J. Brower, 1958 b.
Parides neophilus	Troidini	Blue Jay					X	Present paper.
Danaus p. plexippus (L.)	Danaini	Scrub Jay					X	J. Brower, 1958 a.
Danaus p. megalippe	Danaini	Blue Jay					X	Present paper.
Danaus gilippus berenice (Cramer)	Danaini	Scrub Jay					X	J. Brower, 1958 c.
Lycorea ceres	Lycoreini	Blue Jay			X			Present paper.
Ithomia drymo pellucida	Ithomiini	Blue Jay				X		Present paper.
Heliconius numata Godart	Heliconiini	Silverbeak					X	Brower, Brower, & Collins, 1963.
Heliconius melpomene	Heliconiini	Silverbeak					X	Brower, Brower, & Collins, 1963.
Heliconius erato	Heliconiini	Silverbeak					X	Brower, Brower, & Collins, 1963.
Heliconius sara	Heliconiini	Silverbeak					X	Brower, Brower, & Collins, 1963.
		Blue Jay			X			Present paper.
Heliconius doris (L.)	Heliconiini	Silverbeak				X		Brower, Brower, & Collins, 1963.
Dryas iulia (Fabr.)	Heliconiini	Silverbeak				X		Brower, Brower, & Collins, 1963.
Agraulis vanillae (L.)	Heliconiini	Silverbeak				X		Brower, Brower, & Collins, 1963.
		Blue Jay		X				Present paper.
Biblis hyperia	Ergolini	Blue Jay		X				Present paper.
Anartia amalthea	Vanessini	Blue Jay	X					Present paper.
Victorina steneles	Vanessini	Blue Jay	X					Present paper.
Limenitis a. archippus (Cramer)	Limenitini (Nymphalinae)	Scrub Jay			X			J. Brower, 1958 a.
Euptychia spp.	Satyrini	Silverbeak	X					Brower, Brower, & Collins, 1963.
		Blue Jay		X				Present paper.

species. This same tendency was also evident in another investigation where Scrub Jays freely ate *Papilio glaucus* Linnaeus, uniformly rejected *Danaus plexippus* (Linnaeus), and treated *Limenitis archippus archippus* (Cramer) variably, but as a group ate about half of them (J. Brower, 1958a). Since this appears to be a general phenomenon, it must be discussed further. Let us first investigate the possible reasons for the variable treatment and then consider its bearing on Batesian and Müllerian mimicry.

There are at least four explanations. First, individual butterflies could differ in their palatability through genetic polymorphism or continuous variation. There is no evidence for or against such genetically controlled differences but there are theoretical grounds for supposing that the scent of some species is polymorphic (Rothschild, 1961; Ford, 1964). F. Müller (1878) has noted a dimorphism of odor in the males of *Battus polydamus* (Linnaeus) and insofar as taste is partially dependent upon scent, dimorphism in flavor might also result. However, Müller's finding and palatability differences in general could also be explained (2) by environmentally determined differences among the individual butterflies, depending, for example, on the species of foodplant which their larvae had ingested in nature. There are several species of *Passiflora* available for the heliconiines in Trinidad (Alexander, 1961), and it is probable that *Lycorea* feeds upon more than one of the numerous Asclepiadaceae plants listed by Williams & Cheesman (1947). (We have observed it ovipositing and have reared several larvae to the adult stage on *Gonolobus broadwayi* Schltr., although several others given *Asclepias curassavica* Linnaeus under identical experimental conditions all died, L. Brower, unpub. data).

There is also the possibility that the birds differed in their taste sensitivity for either (3) genetic, or (4) environmental reasons. There is some critical evidence available that animals respond variably in their feeding preferences under experimental conditions. Kare & Ficken (1963) have shown that two-month-old domestic chickens exhibit marked differences in taste thresholds for various salt solutions, although importantly, irrespective of sex. They also discussed selective breeding experiments with chickens which indicated that the variation is under polygenic control. This seems true for saccharin preference in the rat as well (Nachman, 1959). Other experiments have shown variability, but have not determined whether it was genetically or environmentally controlled. Thus J. Brower (1960) found that some Starlings, *Sturnus vulgaris* Linnaeus, rejected food treated with a particular concentration of quinine dihydrochloride, while others accepted it. Similarly, some toads, *Bufo terrestris* (Bonnaterre), freely ate stinging honeybees while others quickly learned to reject them (Brower & Brower, 1962). Other instances of differences in individual behavior towards identical food or flavored water solutions have been reported by Kare (1961) for the domestic chicken and game pheasant, by Kitchell (1961) for domestic pigeons, and by Duncan (1960a) for the feral pigeon. The latter author also found that certain pigeons are "taste-blind" to solutions which are normally repellent to other individuals (Duncan, 1960b; 1961). It is also clear that external and internal environmental differences can affect individuals' food preferences. Aside from the established fact that the rejection threshold for various noxious substances drops as the hunger of the animal increases, it is known that taste thresholds in both mammals and frogs vary with the temperature of the food as well as with atmospheric pressure (Moncrieff, 1951; Sato, 1963). Moreover, as Pfaffman (1963) has shown for rats, sensitivity towards food substances can be manipulated by what they are fed as well as by upsetting their hormone balance. Duncan (1960a, and b) also stated that hand-reared, naive, and wild-captured feral pigeons exhibit different taste preferences.

In the present study, it seems unlikely that the Blue Jays' taste thresholds varied for environmental reasons, because they were all maintained under comparable feeding and experimental conditions. Until further research is done, it does not seem possible to differentiate between the four alternatives. However, it is important to settle this matter because it may be the key to solving the problem of why certain presumably Müllerian species are polymorphic when theory predicts that they should be monomorphic so as to reduce the number of color-patterns that have to be remembered by the predators (Ford, 1964; Sheppard, 1963). Species of intermediate palatability may be so close to the threshold of being acceptable that under certain circumstances (*e.g.*, the dry season, or other periods when alternative prey are less available) they may be taken, while at other times when food is more abundant they may be rejected. Consequently, they could alternate between being Batesian and Müllerian mimics, and if the former situation on the average prevailed, polymorphism would thereby be selectively advantageous.

5. Speculations on the Evolution of Unpalatability

Discussions of protective devices in animals generally assume that they are products of specialization. Thus palatability is considered primitive and unpalatability specialized. It seems certain that this mode of defense has arisen independently several times in the butterflies, as is indeed suggested by perusal of Table 1. Moreover, it is probably still developing in certain lines, as Wallace (1889) suggested when discussing the Pieridae.

In this regard, the findings for *Lycorea* are of particular interest. This species is one of many belonging to the tiger-stripe complex in the neotropics which includes members of three groups, the Lycoreini, the Ithomiinae and the Heliconiinae (Kaye, 1906). It has always been maintained that these are all unpalatable and comprise a Müllerian relationship. This is supported by our findings, but we expected *Lycorea* to be as unpalatable as the others in these groups, which it is not. It is thus possible that the Lycoreini, the most primitive tribe of the Danainae (Forbes, 1939), are still in the process of evolving unpalatability and their mimicry of the other tiger groups is neither Batesian nor Müllerian but somewhere between these extremes.

On the other hand, it seems possible that *Lycorea* may represent an instance of the regression from unpalatability back towards palatability. The evolution of unpalatability must require complex readjustments in the physiology of a species so that the noxious chemical substances can be accommodated without poisoning the animal's own system, as discussed below (Section IV-D). Furthermore, even after the mechanisms have evolved, it is likely that they are to a certain extent detrimental to their possessors. Consequently, large selective forces favoring unpalatability must be required not only for its evolution, but also for its maintenance. Therefore if the advantage of unpalatability should lessen for any reason, it is possible that the genes controlling its development will be replaced by those which no longer produce it. This may have occurred for *Lycorea* as a consequence of the great number of unpalatable ithomiines and heliconiines which share its color-pattern. In this situation a species such as *Lycorea* could lose its unpalatability but retain the protective advantage of mimicry, particularly if it were rare. In other words, if the environment becomes saturated with many similarly colored, unpalatable species, the balance of advantage for unpalatability could be decreased to the extent that selection would favor a reversal from a Müllerian towards a Batesian status.

One way to test these alternatives is to study the species of the genus *Ituna* which are closely related to *Lycorea,* but are not involved in the tiger mimicry complex, and the species of the genus *Clothilda* which are close to the nymphalid stem from which the other two genera have evolved. If *Lycorea* is more palatable than its close relatives but more unpalatable than the ancestral group, then regressive evolution is suggested. If they are about equally unpalatable and both less palatable than the ancestral group, then unpalatability is probably still in the process of evolution.

More generally, whereas regression of unpalatability may occur this way in certain of the exceptional Müllerian mimicry complexes, it probably occurs rarely in nature because of continual predation pressure. This is in fact supported by our findings that the unpalatability of species generally corroborates morphologically-based speculations on phylogeny. The theoretical interest of these possibilities associated with species of intermediate palatability emphasizes the dynamic relationship of Batesian and Müllerian mimicry and makes further research greatly to be desired.

IV. THE EVOLUTION OF PLANT POISONS AND THEIR BIOCHEMICAL FATE WHEN INGESTED

(A). *The General Problem*

Within ecological communities, there are three important ways in which the non-nutrient chemicals produced by plants may affect the animals. First, there is a large body of indirect evidence that many of these compounds have evolved as repellents to phytophagous insects and probably to other herbivorous invertebrates. Moreover, some of these repellents in the course of evolution appear to have been exploited secondarily as signal attractants and so operate in the physiological regulation of foodplant specificity (Dethier, 1954; L. Brower, 1958; Fraenkel, 1959; D. A. Jones, 1962).

Secondly, numerous plant components are known which are toxic or repulsive to herbivorous vertebrates. While there is a vast literature on the pharmacology, toxicology and medicinal effects of these chemicals on man and other mammals, there is little more than cursory discussion of the theory that (2) poisonous plant products have evolved as chemical repellents in response to the selection pressures exerted by all herbivorous vertebrates. Thus, the excellent discussion by Gundersen & Hastings (1944) on interdependence in plant and animal evolution did not develop the idea of this chemical relationship and this is true of the literature that we have examined in both general and specific fields of plant and animal ecology, evolution, psychology, physiology and pharmacology[3].

The third interaction involves three links in the foodchain—plants, herbivores and predators—rather than just plants and herbivores as in the first two interactions. The question is (3) have herbivorous vertebrates and invertebrates in the course of their evolution developed the ability to incorporate the poisonous and repellent plant chemicals, which have evolved as herbivore deterrents, and in turn use them to deter their own predators? The recent paper by Rothschild (1964) has come the closest to presenting this idea, but as far as we can determine, it has never been completely formulated in the literature; its proof will establish a new principle of biology. The remainder of this paper will attempt to elucidate various aspects of this third interaction, as exemplified by some phytophagous insects.

(B). *Evidence that the Larval Foodplants of Unpalatable Butterflies Contain Poisons*

Before we consider the mechanisms by which insects might utilize the secondary plant substances as part of their own defense against vertebrate predators, the literature will be reviewed to show that the larval foodplants of the five unpalatable groups of butterflies contain poisonous substances. These belong, as do many non-poisonous components, to a large class of compounds known as glycosides (Allport, 1943; McIlroy, 1951; Robinson, 1963). Glycosides consist of two major conjugates which, when hydrolyzed, break down to a sugar and an aglycone. In those that are poisonous, the aglycone may have quite different toxic effects from the glycoside itself. As will be seen below, the poisonous glycosides found in the foodplants of the unpalatable butterflies fall into four main categories: (1) *cyanogenic glycosides,* which on hydrolysis yield a cyanide-containing aglycone which in turn breaks down to form HCN; (2) *cardiac glycosides* (cardenolides), in which the aglycone (specifically called a genin) is a steroid that lacks nitrogen; (3) *saponins,* another special class of glycosides in which the aglycone is either a triterpenoid alcohol or a spiroketal steroid; (4) *alkaloids,* a heterogeneous group of usually basic compounds often occurring as glycosides in which the aglycone is a nitrogen-containing steroid.

Evidence that these poisonous substances occur in species from the various families to be discussed is based on a variety of source material ranging from crude chemical tests in the field and information collected by naturalists on the uses of plants by local natives, to highly critical analyses carried out in modern laboratories. It is well to remember that the original discovery of medicinally valuable plants has depended principally upon their producing extreme physiological effects. Thus while repulsive, sickening or poisonous if eaten in quantity or without special preparation, when administered in sufficiently dilute concentration, they are either useful or at least produce pseudo-medicinal results. The poisonous properties of these plant substances are based largely upon their effects on mammals, and proof that they provide the chemical source of unpalatability in the insects awaits further investigation.

The Aristolochiaceae

According to Willaman & Schubert (1961), plants in this family contain at least ten different alkaloids, one of which, aristolochine, is said to cause cardiac and respiratory paralysis in mice as well as frogs (Henry, 1949). *Aristolochia rumicifolia* Mart. & Zucc. is used to make a curare arrow poison in South America (Henry, 1949). In India, members of the family are said to be nauseous and abortifacient (they are commonly called the birthworts), and are also used as insecticides (Chopra & Chopra, 1955). In Africa, the roots of one species, *Aristolochia albida* Duchartre, are made into a weak, cold infusion and taken as a tonic, while the leaf is used for treatment of skin diseases and mixed with other components and drunk (Dalziel, 1937).

The Solanaceae

Even though plants of this family provide edible vegetables (potatoes, tomatoes and eggplant), they are generally narcotic and poisonous because of their contained alkaloids. These include the well-known and pharmaceutically important parasympathetic depressants from *Atropa belladonna* Linnaeus, as well as soladine and solanine from *Solanum,* and nicotine from *Nicotiana* (Allport, 1943; Chopra & Chopra, 1955; Willaman & Schubert, 1961). The poisonous properties of solanine were studied by Kline, von Elbe, Dahle & Kupchan (1961) who found that pregnant rats which had eaten sprouts of potato tubers gave birth to dead young or those which died in less than three days. The same effects were obtained when pure solanine was added to their diet, and it was concluded that the sprouts contained this alkaloid. Watt & Breyer-Brandwijk (1932) described the clinical effects of solanine which include vomiting, headache, diarrhea and colic, followed by depression. It is possible that the leaves of some species are edible, as Grisebach (1864) mentioned that *Solanum nodiflorum*

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[3] Investigations of the physiological and psychological functions of the sense of taste in vertebrates could benefit greatly by being more cognizant of this ecological interaction with the plants (see Richter 1957; Teitelbaum & Epstein, 1963).

is eaten as a spinach, although Willaman & Schubert (1961) indicated that the fruits of this species contain solasodine. The fact that plants can be eaten as spinach may well result from the fact that the poisons in the leaves are extracted in the cooking water.

The Asclepiadaceae

These plants and their near relatives, the Apocyanaceae, are renowned for their numerous highly toxic cardiac glycosides (Abisch & Reichstein, 1962; Paris, 1963). Tschesche, Forstmann & Rao (1958) have isolated and partially identified seven different genins from *Asclepias curassavica.* This plant is native to tropical America, and in Trinidad we have reared several hundred larvae of *Danaus p. megalippe* and *D. gilippus xanthippus* Felder to the adult stage on it. According to Tschesche *et al.*, the usual extract from this plant is made from dried leaves and young parts of the stems, *i.e.*, precisely the parts which the larvae eat. Used in an infusion for pharmacological purposes, this acts as an excitant, an expectorant and a regulator of blood pressure, while the root extract is used as an emetic and a purgative. The *Asclepias* species in addition contain alkaloids as well as saponins (Henry, 1949; Chopra & Chopra, 1955). Whereas cardiac glycosides and their genins act, respectively, by affecting the heartbeat, and by causing convulsions, the general action of saponins is to irritate mucous membranes and to haemolyse red blood corpuscles when injected into higher animals. They act as highly toxic environmental poisons to fish (McIlroy, 1951). The aglycones of steroid saponins are also convulsants. Evidence of these toxic effects was given by Chopra & Chopra (1955) who stated that *A. curassavica* is used in India as a fish poison, an emetic and a cathartic. In Africa, Irvine (1961) noted that this species contains an active principle which causes death in large doses and is almost certainly poisonous to cattle.

Moreover, nicotine, a solanaceous alkaloid also found in *Asclepias* species, has an action on nerve ganglia resembling that of acetylcholine in producing a strong initial stimulation of the receptor, but differing in that the resultant depolarization is greatly protracted. Thus a temporary paralysis ensues (Cutting, 1962). Nicotine has therefore proved useful in the capture of wild animals. It is of interest in this context that in West Africa, Gilges (1955) reported that the natives in Balovale sprinkle the roots of *Asclepias lineolata* (Schltr.) among grain in order to catch birds, which become unconscious after eating them. On the other hand, Dalziel (1937) stated that the roots of the same species are used in an infusion to treat gastro-intestinal ailments. This finding again emphasizes that although the plants are poisonous, they may be used medicinally if prepared and administered in sufficiently low concentrations. In addition, the young leaves, pods and shoots, as well as the tubers of some species of North American *Asclepias,* appear to be edible (Sturtevant, 1919; Watt & Breyer-Brandwijk, 1932). But as above, this is probably because the poisons are extracted in the cooking water. Another genus, *Calotropis,* is used to cause abortion, in homicide and suicide, and its "milk" is a drastic purgative (Chopra & Chopra, 1955). Tschesche, Snatzke & Grimmer (1959) have established that the genin from *Calotropis procera* known as calotropagenin is identical to asclepogenin from *A. curassavica.*

One of the best known members of the apocyanid group is the genus *Rauwolfia* which contains numerous poisonous alkaloids, including the tranquilizing drug, reserpine (Woodson, Youngken, Schlittler & Schneider, 1957; Irvine, 1961; Willaman & Schubert, 1961). Another apocyanid, *Nerium indicum* Mill., is used for suicide and abortion. In addition, *Nerium oleander* Linnaeus contains the glucosides neriin and oleandrin and is fatal to horses and cattle and to man even from meat skewered with the wood (Watt & Breyer-Brandwijk, 1932). Irvine (1961) has noted that many *Adenium* spp. are used in Africa to poison jackals and hyenas by baiting them to meat filled with a concoction of the plants. They are also used in arrow poison and to kill fish. These varied uses suggest the action of more than one type of poison, probably the cardiac glycosides in the baiting of mammals, and saponins for fish and poison arrows.

The Passifloraceae

These plants have been cited as non-poisonous by Poulton (1916), Sevastopulo (1952), and Brower, Brower & Collins (1963). Moreover, Henry (1949) did not include them in his book on alkaloids, nor did Muenscher (1939) in his book on poisonous plants. *Passiflora foetida* Linnaeus has edible fruit as do 16 other species of *Passiflora,* according to Sturtevant (1919). In fact, the leaves of *P. foetida* and its fruit when green contain cyanogenic glycosides as well as alkaloids and may be dangerous to stock (Dalziel, 1937; Arthur, 1954). Willaman & Schubert (1961) have in addition listed the alkaloid passiflorine from seven species, and Chopra & Chopra (1955) noted that this family produces hydrocyanic acid. Two species, *Adenia palmata* Engl. and *A. digitata* Burtt Davy, have poisonous roots and fruits due to the presence of a cyanogenic glycoside and the toxoalbumin, modeccin, (Watt & Breyer-Brandwijk, 1932). Another species, *Adenia lobata* (Jacq.) Engl., is said to be used in arrow poison (Irvine, 1961). Thus the statements that the Passifloraceae are harmless were incorrect and the fact that the fruits are in some cases edible recalls the potato tuber which, too, is an unpoisonous part of an otherwise deadly plant.

From this survey, it can now be considered established that many poisonous chemicals occur in the plant families fed upon by the five major taxa of unpalatable butterflies. However, just as parts of these plants are sometimes edible, it is well to remember that most other plant families contain species, parts of which at some stage in their development contain poisonous substances (Willaman & Schubert, 1961). This includes some of the families fed upon by the palatable species investigated in this paper. The cited foodplant of *Biblis, Tragia volubilis* Linnaeus, is in the Euphorbiaceae and was considered by Grisebach (1864) to have an acrid and poisonous milky juice. The leaves of other species apparently produce hydrogen cyanide and according to Arthur (1954) may contain alkaloids. However, Webb (1955) studied 22 species from New Guinea in which no alkaloids were present and stated that plants of this family are certainly of negligible alkaloid interest. Many genera have multiple medicinal uses, and only the latex is stressed as a dangerous poison, while fruits and leaves are often eaten by antelopes and other animals (Irvine, 1961). Similarly palatable is the widely eaten rhizome, cassava, which comes from another member of this family, *Manihot esculenta* Crantz., although the fresh tubers are said to be extremely poisonous, and cassava has to be specially prepared before it is safe to eat (Chopra & Chopra, 1955).

Some of the plants belonging to the Acanthaceae, which contains the species eaten by *Victorina* and *Anartia*, are said to produce bitter or aromatic drugs (Grisebach, 1864) and others contain alkaloids (Arthur, 1954), while still others are also used medicinally (Irvine, 1961). On the other hand, the leaves of three genera are eaten as spinach and are not considered poisonous (Irvine, 1961). Furthermore, the Graminae, the foodplants of *Euptychia* spp., are generally edible to herbivores, but Chopra & Chopra (1955) have noted that some species of grass develop large quantities of hydrocyanic acid during dry periods.

It thus appears from this survey that the plant families of the palatable butterflies are not distinguished for poisonous properties, although exceptions exist, whereas among the foodplants of unpalatable butterflies, the reverse is true, most being poisonous. Clearly, more critical evidence is needed, and while these generalizations about plant families are valuable, quantitative and qualitative analyses of the particular species of plant fed upon by the insects must be made. Furthermore, the actual parts of the plants eaten must be analyzed, and these should be taken from the plants at a time which corresponds to the natural feeding period of the insects. For as Flück (1963) has pointed out, secondary substances may be synthesized in one part of a plant but concentrated in another, and variation in the amounts of the chemicals in any one tissue occurs seasonally, daily, and according to the physiological state of the plant.

(C). Utilization of the Plant Poisons

There are several ways in which ingested poisonous plant substances might be utilized as predator deterrents. Let us consider these for the phytophagous insects in order of increasing complexity. Defense could result simply by (1) *the accumulation of the plant chemicals in the gut.* This idea was first tested by Eltringham (1909, 1910) who found that larvae of the moth *Odontopera* (= *Gonodontis*) *bidentata* Clerck were extremely distasteful to lizards, *Lacerta viridis,* when fed ivy, but when subsequently given apple for a period of time, became palatable. Recently Eisner, Kafatos & Linsley (1962) have extended this to a fourth link in the foodchain by suggesting that cerambycid beetles of the genus *Elytroleptus* may become temporarily unpalatable by eating lycid beetles, *Lycus* spp., which themselves are distasteful. This mechanism could suffice for insects which continue throughout their lives to eat poisonous foods, but it would not account for the unpalatability of those such as adult Lepidoptera, which undergo a radical change in food habits after metamorphosis, either shifting to nectar or ceasing to eat altogether.

Consequently, an alternative mechanism is required which could be (2) *the assimilation and storage of poisonous molecules generally throughout the blood or other body tissues.* Meldola (1877) first suggested this and it is still held possible by modern workers (Eisner, Kafatos & Linsley, 1962). The first support came from the work of F. M. Jones (1937). Using three species of *Asclepias*-eating beetles, *Tetraopes tetraophthalmus* Frst. (Cerambycidae), and *Chrysochus auratus* Fabr., and *Labidomera clivicollis* Kby. (Chrysomelidae), he found that individuals which had recently been fed the plant and those which had been starved for five days to clear the gut were rejected by both birds and ants, even though these predators ate control food items. A more direct line of evidence was produced by D. A. Jones, Parsons & Rothschild (1962) who showed that the tissues in all stages of two species of zygaenid moths produce hydrocyanic acid when crushed, and a precursor of this poison, a cyanogenic glucoside, is also present in their foodplants. However, this example is complicated by the fact that the same insects produce HCN when fed upon an acyanogenic strain of the same plant, as will be discussed in Section IV-E.

The most specialized mechanism could be (3) *the assimilation, concentration and storage of the poisonous molecules in glands.* Roth & Eisner (1962) agree with the probable existence of this mechanism, but there are in fact no certain demonstrations of it. The most likely example comes from the work of Waterhouse, Forss & Hackman (1961) who have shown that a coreid stink-bug, *Amorbus rubiginosus* Guer., produces 2-hexenal, an aldehyde identical or chemically similar to that found in several plant species including *Eucalyptus* upon which they feed. It is almost certainly defensive since it is effective in repelling ants in the laboratory, and related but unidentified pentatomid and coreid bugs deter ants, amphibia, birds and mice. The cockroach, *Eurycotis floridana* (Walker), also produces 2-hexenal (Roth, Niegisch & Stahl, 1956), as do two other phytophagous bugs, *Brochymena quadripustulata* (Fabr.) and *Acanthocephala femorata* (Fabr.) (Blum, Crain & Chidester, 1961; Blum, 1961). However, once again the evidence that they concentrate the aldehyde from their food is only correlative. Another possible case was discussed by Pavan (1959). He proposed a biochemical scheme by which salicyl aldehyde from the dorsal gland of the beetle larva, *Melasoma populi* Linnaeus, might be constructed from components present in its foodplant, willow (Salicaceae).

Less direct evidence that the insects utilize the plant substances also exists. Two highly pertinent studies on *Asclepias*-feeding grasshoppers have been made. Steyn (1962) reported detailed observations on the death of a four-year-old Bantu child within 12 hours after she had eaten a single adult *Phymateus leprosus* Fabr. Symptoms included extreme vomiting and salivation, followed by respiratory and cardiac failure. In subsequent experiments with adult rabbits, he found that 90 grams of freshly minced grasshoppers suspended in water and fed by stomach tube also caused respiratory and cardiac deterioration and death. In nature this species is known to feed on *Asclepias fruticosa* and *Nerium oleander.* More recently Parsons (1963, and personal communication) has reported that the secretion of one individual of *Poekilocerus bufonius* Klug is sufficient to kill several cats when injected intravenously. The poison, which he is investigating (Rothschild & Parsons, 1962), appears to be digitalis-like in its action and may be a cardiac glycoside similar to that found in its foodplant, *Asclepias curassavica.*

An additional line of indirect evidence has been cited by McCann (1953) who described an Indian grasshopper, *Aularches miliaris* (Linnaeus), which as nymphs eat the pungent *Heterophragma roxburghi* A. de C. (Bignoniaceae) and emit an odor similar to that of the leaves. As adults, they change their feeding habits and eat the highly poisonous and acrid leaves of *Gloriosa superba* Linnaeus (Liliaceae), and now emit a fluid with a corresponding odor.

Finally, the correlation between the unpalatable butterflies and their poisonous foodplants has two remarkable parallels in the Hemiptera of the New World. The lygaeid bugs, *Onchopeltus fasciatus* (Dallas) and *Lygaeus kalmii* Stal., feed upon *Asclepias* and are highly distasteful to lizards, Scrub Jays and ants (F. M. Jones, 1932; Parsons, 1940; J. Brower, unpub. data). In Trinidad two warningly colored and gregarious coreids, *Leptoglossus (Diactor) bilineatus* Fabr. and *Anisoceles (Hymeniphera) hymeniphera* Westwood, live upon *Passiflora serrato-digitata* Linnaeus which they almost certainly eat (Brower & Brower, personal observations, summer, 1963).

(D). *Mechanisms for Preventing Autotoxicity*

In order for herbivores to utilize the plant poisons in their own defense, they must have mechanisms to prevent themselves from being poisoned, as is indeed implied by their very ability to feed upon the plants containing these substances. A large body of biochemical evidence has shown that both vertebrates and invertebrates are able to transform poisonous molecules into non-poisonous ones either when taken orally or injected. Detoxification more often than not results in increasing the size of the molecule rather than in breaking it down, and the products can be accumulated in a harmless form in the tissues of the animals, as well as excreted or defecated (Smith, 1955; Brodie, 1956; Gilmour, 1961).

If the defense were simply by accumulation in the gut (method 1 above), no metabolic reworking of the poisons would be required, provided that the insect itself were resistant to the chemical. In this instance the plant poison would be transferred directly to the predator. On the other hand, modern findings in the field of detoxification biochemistry as discussed by Williams (1959) make it more likely that the substance would be built into a harmless compound in the gut, but would break down again when ingested by a predator. Similarly detoxified compounds could be distributed throughout the body tissue or hemolymph (method 2), or concentrated in glands (method 3) and again break down to yield the active poisons when eaten by the predator.

The recent study of Remold (1963) is of interest in showing how autotoxicity is avoided by mechanical means in the pentatomid bug *Carpocoris purpureipennis,* which secretes a defensive aldehyde in specialized glands. When sprayed by the bug on ants or other insects, this substance immediately flows over their cuticular surface and acts as a contact poison, causing paralysis. Moreover, it also repels them from a distance. Puncturing the reservoir of an individual bug resulted in releasing the secretion into its hemolymph, which caused immediate paralysis, as did allowing it to flow over the surface of the bug's body after abrading the cement layer. Thus these bugs' own chemical defense system is highly poisonous, but is kept harmless by storage in the reservoir gland, and by impermeability of their intact cuticle. Moreover, entrance of the substance into their own tracheae is prevented by specialized mushroom-shaped cuticular structures near their spiracles, which greatly increase the evaporative surface.

Another instance where a defensive secretion of an insect has been proven autotoxic is in the case of the cockroach, *Eurycotis floridana,* mentioned above (Roth, Niegisch & Stahl, 1956). Similarly, Waterhouse, Forss & Hackman (1961) showed that the coreid stink-bug, *Rhoecocoris,* dies if the insects are crowded together without ventilation. In addition, some insects synthesize toxic substances *de novo* which they store in a harmless form through conjugation with glucose to form beta-glucosides (Brunet, 1963a, b; Brunet & Kent, 1955). Other examples of autotoxic secretions are given by Pavan (1959), Crescitelli & Geissman (1962) and Roth & Eisner (1962).

It is important to realize that some species of phytophagous insects which feed upon poisonous plants are cryptic and for this reason have been assumed to be palatable (Sevastopulo, 1952). It is possible that some of these are adapted to destroy, excrete, defecate or convert the poisons into permanently detoxified compounds rather than secondarily utilizing them to build up their own chemical defense systems. Others may still be in the process of evolving unpalatability as discussed in Section III-D-5, and may not yet have evolved a warning coloration. However, some species, such as the larvae of the moth *Mania* (= *Phalaena*) *typica* Linnaeus, are both extremely unpalatable and highly cryptic (Poulton, 1887; Eltringham, 1909). Consequently, cryptic coloration should not always be interpreted to indicate palatability any more than conspicuous coloration always implies the reverse, as for example, in Batesian mimics, or in species such as the moth, *Rhododipsa masoni* J. B. Smith, which, though yellow and scarlet, inhabits similarly colored flowers and is therefore in fact cryptic (Brower & Brower, 1956).

Finally it is of great interest that insects differ basically from vertebrates, but are similar to plants, in the way in which they detoxify certain natural products by conjugation with sugar or sugar derivatives: vertebrates form glucuronic acids or glucuronides, while insects and plants form glycosides (Williams, 1959; Pridham, 1960). This suggests that the biochemical machinery of the insects is closely adapted to that of plants and in our opinion increases the likelihood that the insects can utilize the poisons from the plants rather than having to produce them by *de novo* synthesis.

(E) *The Alternative of* de novo *Synthesis of the Defensive Poisons*

Roth & Eisner (1962) have suggested that the defensive secretions of most arthropods are probably produced metabolically, that is to say, through *de novo* synthesis. For example, the quinones are widely used in defense, and as Brunet (1963a, b) has shown, these most often seem to be synthesized from the amino acids tyrosine and phenylalanine. The production of hydrocyanic acid in zygaenid moths may also be metabolic, instead of derived from the plant as discussed above. Even when fed upon strains of plants in which the cyanogenic glycoside has not been detected, the moths still produced it when their tissues were crushed (D. A. Jones, *et al.,* 1962). It seems to us that more work is needed on this problem, for the reason that the glycoside may occur in the plant in such a low concentration as to be difficult to detect, and yet be concentrated by the insects. A similar criticism can be directed towards another elegant investigation. In attempting to shed light on the biochemical origin of aldehydes in the Hemiptera, Gordon, Waterhouse & Gilby (1963) injected green vegetable bugs, *Nezara viridula* (Fabr.) (Pentatomidae), with radioactive sodium acetate. The purpose of the experiment was to find out whether or not the species could utilize the acetate molecule as a building unit in the synthesis of the aldehydes and thus prove that the repellent substance need not be concentrated from the plants. The results indicated beyond doubt that the radioactive carbon ended up in the bugs' repellent secretion. However, the experiment by-passed the digestive system and does not disprove that the bugs may be able to incorporate the aldehydes from the plants they eat.

Although the idea of *de novo* synthesis, originally put forward by Meldola (1877), is of undoubted importance, we suspect that further research will establish that the poisonous molecules in the plants are more often utilized by the insects in biochemical defense than is at present thought to be the case. This prediction is made more plausible when one considers that carotenoids are synthesised only by plants, yet are often in the integumental coloring of insects (Cromartie, 1959; Fox & Vevers, 1960) and form the biochemical basis of the visual systems of both vertebrates and invertebrates (Gilmour, 1961; Prosser & Brown, 1961).

V. CONCLUSION

While correlation does not prove causation, we believe that the facts, as presented in this paper, make a casual relation almost certain, *i.e.*, the adult butterflies of five major taxa are unpalatable to vertebrate predators because their larvae are able to incorporate into their own tissues the unpalatable and poisonous molecules produced by the plants.

One approach to obtaining proof of this relationship would be to offer to vertebrate predators the adults of a polyphagous butterfly species whose larvae had been fed either poisonous or non-poisonous foodplants. Analyses could then be undertaken if a significant difference were found between the two groups. A species which appears to be particularly suitable is *Euptoieta claudia* Cramer, a member of the tribe Argynnini near the nymphalid stem, which on the one hand has given rise to the palatable nymphalines and on the other to the unpalatable heliconiines. This butterfly is reported to feed on plants from a number of families, including the Violaceae which are the main food of the palatable argynnids, and the Passifloraceae, the foodplants of the heliconiines and acraeids. Indeed, Hasse (1896) suggested that the most primitive heliconiine genera have become unpalatable by changing their food from related families such as Violaceae to the Passifloraceae. A second approach would be to induce a known unpalatable species to eat a non-poisonous foodplant and determine if it then becomes palatable. For example, the milkweed bug, *Onchopeltus fasciatus*, can be fed a variety of edible seeds including raw peanuts (Frings & Peissner, 1952). Alternatively, synthetic diets could be used (see House, 1961).

With the analytical tools now available in biology, including chromatography and labelling with radioactive substances, the study of the transfer of chemical molecules from the plant through the herbivore has become an exciting prospect. It will be a fitting reward to the efforts of naturalists over the past hundred years if these new methods prove their speculations correct.

VI. SUMMARY

1. Representative species from four of the five major groups of butterflies (Troidini, Heliconiinae, Danainae and Ithomiinae) classically held to be unpalatable to vertebrate predators were quantitatively demonstrated to be unacceptable to five experimental Blue Jays, *Cyanocitta cristata bromia,* and those from two other groups (Satyrinae and Nymphalinae) held to be palatable proved to be so.

2. The foodplants of the distasteful groups of butterflies belong, respectively, to the families Aristolochiaceae, Passifloraceae, Asclepiadaceae and Solanaceae. These are all characterized by their contained poisonous substances and contrast with the foodplants of the edible groups of butterflies which in general are not poisonous.

3. A large body of correlative evidence is presented in support of the theory that plants which contain substances repellent or toxic to herbivorous vertebrates and invertebrates impart to specific phytophagous insects their poisonous molecules which these insects in turn utilize in their own defense against vertebrate predators.

4. It is thus thought that the specific chemical substances which cause adult butterflies to be unpalatable are derived from the poisons in the larval foodplants rather than synthesized *de novo* by the insects.

5. The experiment established that comparative palatability of species ranks with morphology in assessing phylogenetic relationships.

6. The Blue Jays treated both the very palatable and the very unpalatable species uniformly, whereas individual birds tended either to eat or reject those of intermediate palatability. This variable treatment of the intermediate species is interpreted as a threshold phenomenon and may explain why certain Müllerian mimics are polymorphic.

7. The evolution and maintenance of unpalatability, which is of advantage in reducing predation, is considered to be physiologically detrimental. Consequently, in certain species of large Müllerian associations, the balance of advantage for unpalatability could be decreased to an extent that selection would favor a reversal from a Müllerian towards a Batesian status.

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10

Protective Display and Sound Production in Some New World Arctiid and Ctenuchid Moths[1]

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(Text-figures 1-29)

1. Introduction

Despite a large literature on the protective devices of arthropods, there have been few attempts to study their evolution within restricted taxa in relation to particular natural communities. Such studies are to be desired because the growth of knowledge about the behavior of predators in prey/predator situations (see reviews by de Ruiter, 1956; Sheppard, 1959, 1961) has led to a belief that the selection pressures which determine the evolution of protective behavior may be formulable in fairly simple terms, and that the end-products of evolution under given circumstances may be to some extent predictable. For instance, a discussion of Müllerian and Batesian mimicry based on assumptions of this type has been given by Huheey (1961), and of the constraints which may modify the adult lifespans of some hemileucine saturniid moths by Blest (1963b).

Nevertheless, it may be doubted whether many natural situations present a sufficient degree of simplicity for such analyses, and the present more superficial survey of a large fauna may serve to illustrate some of the difficulties of such an approach.

The protective display behavior of arctiid and ctenuchid moths will be described in three main neotropical locations: at Barro Colorado Island, in the Panamá Canal Zone (lowland seasonal tropical evergreen forest: Bennett, 1963); at Volcán and Santa Clara near the Panamá-Costa Rica border (seasonal lower montane forest at 4,000-5,000 ft. above sea level); and in the Arima valley of Trinidad (transitional between lower montane rain forest and lowland seasonal forest, at an effective altitude of 500-1,800 ft. above sea-level (Beard, 1946; Beebe, 1952).

These two families were chosen because they form a group of allied, numerous and predominantly distasteful species, a large proportion of which have been accessibly monographed for the areas concerned (Forbes, 1939; Kay & Lamont, 1937; Fleming, 1957, 1959).

The nomenclature follows that used in the current arrangement of material in the British Museum (Natural History) collections, and it may be noted that Forbes (1939) lists illustrations in Seitz (1915-17) for most of the species which he records for Barro Colorado and comments on their accuracy.

Although the ctenuchid fauna is a rich one, this group has not been studied in as much detail as the arctiids; a sufficient range of species has been examined to establish the general behavioral continuity between the two families, which also show a wide range of morphologically transitional forms (Forbes, 1939). The use of light traps to secure material has caused the predominantly diurnal Ctenuchinae to be poorly represented. No attempt has been made to study the behavior of the minute and fragile lithosiine arctiids. The behavior of virtually all the dominant species on Barro Colorado has been examined between May 5-September 20, 1961, July 2-September 20, 1962, and the month of July, 1963; only those species which are rare or whose identity is uncertain are omitted. In Trinidad, only the most common species were seen

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in April, 1961, and work at Volcán and Santa Clara was confined to three weeks in July, 1961.

2. Materials and Methods

The moths were captured as adults at light traps which were run throughout the night; the insects were removed from the vicinity of the light and placed in separate containers as soon as possible after arrival at the traps. The simple methods for examining protective display behavior have been described elsewhere (Blest, 1957).

The palatability tests employed White-faced Monkeys, (*Cebus albifrons*), and Geoffroy's Pinché (*Oedipomidas spix*) (Canal Zone); and domestic fowl (Santa Clara). The monkeys and marmosets were confined in groups of 4-6 in large outdoor cages.

3. Modes of Protective Behavior

The protective devices can be divided into a small number of behavior patterns found widely throughout the two groups, while occasionally showing marked taxonomic segregation, and a larger number of specialized devices with a much more scattered distribution, usually combined in some way with the basic display types. The behavioral repertoire may be summarized as follows:

I. *The Emission of Ultrasonic Signals.*—One species, *Melese laodamia,* has been shown to emit ultrasonic sounds at high intensity when it is roughly handled; the presence of the sound-producing organs on other species, and the emission by them of sounds with a faint audible component, allowed the assumption that they too are primarily generating noises in the ultrasonic range (Blest, Collett & Pye, 1963) and recent observations have confirmed that they do so. The sounds are produced by modified episternites of the metathorax. Those of *Melese* are modulated between 30 and 90 Kc/sec. Each burst of sound is composed of a train of cycles of modulation which sweep over the main frequency range some forty times/second; the modulation cycles themselves are formed from a series of discrete pulses whose repetition rate may rise to over 1,000/sec. The mechanics and acoustics of the process of sound production are discussed in detail elsewhere, together with their possible function. The latter is, however, quite obscure; the thresholds of sound production to tactile stimulation are very variable, and it is not yet clear what proportion of species will emit them while on the wing, or in what stimulating circumstances (Blest, Collett & Pye, 1963).

Table 1 includes a summary of the thresholds at which sounds are produced to handling the insects. Species with a low threshold (L) will start to make sounds as soon as they are picked up and held lightly; those with medium thresholds (M) need to be squeezed, or rolled lightly between the fingers; species with high thresholds (H) must be manipulated to the point of mutilation. Those species marked "VH" perform erratically and only a minority of individuals will respond to mutilation. This classification reveals that (a) any mode of protective coloration *can* be linked to sound production; (b) the advanced mimetic ctenuchids have lost the ability to make sounds; the anatomical condition of the tymbal organs (*i.e.,* their reduction to leathery and densely scaled structures) suggests that they are unlikely to use them in any circumstances; (c) there is within the arctiids, at least, a correlation between threshold of sound production, "toughness," and palatability. This relationship reveals that, predominantly, palatable arctiids have a low threshold for sound emission, while distasteful species are more likely to possess the higher thresholds.

In addition there is another unexplained correlation. While distasteful species with high thresholds for sound production are likely to have their flight periods at any time in the night, palatable species with low thresholds concentrate their flights between 3 and 5 o'clock in the morning. Table 2 summarizes the data for 35 species of arctiid and ctenuchid. The individual moths arriving at an ultra-violet light-trap were recorded hourly and removed from the light and destroyed after they had been scored. The table gives the total number of male individuals for each group of species which were recorded nightly over a three-week period on Barro Colorado Island from June 30-July 25, 1962. It is unlikely that these correlations can be understood until more is known about the emission of ultrasound in free flight, and about the normal flight times of insectivorous bats in the habitats concerned. Several species of arctiid and ctenuchid, including the genera *Melese* and *Halysidota,* can be made to reply with bursts of ultrasound to pulses from a bat simulator (Roeder, unpublished data; Pye, Pye, Flynn & Blest, in prep.), but the significance of this response is not yet understood.

II. *Classical "Reflex Immobilization."*—The use of the term will be restricted, for reasons which have already been discussed (Blest, 1957), to immobilization, or "freezing" which does not involve the assumption of a specialized posture. Characteristically, in both families, it does not outlast actual handling by more than a few seconds, and is given only to a constraining tactile

TABLE 1. LIST OF SPECIES STUDIED, WITH NOTES ON THEIR DISPLAY TYPES, PALATABILITY AND SOUND PRODUCTION

Conventions:

Localities:
B — Barro Colorado
S — Santa Clara and Volcan
T — Arima Valley, Trinidad

Display Type:
R — Reflex immobilization
II — Type II display
III — Type III display
W — Wing-waving display

Sound Production:
L — Low threshold to tactile stimuli
M — Moderate " " "
H — High " " "
VH — Very High " " "

All of the species listed below have been checked against authenticated specimens in the collections of the British Museum (Natural History). Where insufficient specimens were available for valid comparison, or there were no authenticated specimens available, the species is marked ⊣.

ARCTIIDAE PHAEGOPTERINAE (77 species)	Locality	Display type	Additional components, etc.	Sound production	Palatability and notes
Automolis lineosa Walker	B	R	———	L-M	Rejected by *Cebus*
A. lineatus Druce	B	R	———	L-M	" " "
A. sp. (unidentified)⊣	S	R	———	——	Near to *A. lineatus*
A. reducta Walker	B	R	———	H	Rejected by *Cebus*
A. dilutus Felder	B	R	Falls to visual stimuli or touch	H	" " "
A. pauperis Schaus	S	III	———	——	Rejected by fowls
A. rutila Stoll	BT	III	Text fig. 9	H	Rejected by *Cebus*
A. priscilla Rothschild	B	III	———	H	One female only
A. chionoplaga Dognin	B	III	Text fig. 10	H	Rejected by *Cebus*
A. sicilia Druce	BS	III	———	H	" " "
A. metallica Joicey	BT	III	———	H	" " "
A. taeniata Guerin	S	III	Text fig. 11	VH	Rejected by fowl
A. vitrea occidentalis Rothschild	B	III	———	H	Rejected by *Cebus*
A. vinasia Schaus	S	R	———	(–)	Two specimens only
A. elissa Schaus	B	III	———	H	Rejected by *Cebus*
A. elissoides Rothschild	B	III	Text figs. 7 & 8	H	" " "
A. salma Druce	B	III	———	?	One male, Aug. 1963
A. critheis Druce	B	R(III)	———	——	Rejected by *Cebus*
A. tegyra Druce	BS	III	Text fig. 12	VH	" " "
A. obscurata Schaus	B	R(III)	Cryptic color pattern	L	Palatable
Cissura plumbea Hampson	B	R	———	H	Rejected by *Cebus*
Prumala sp. ⊣	S	R	———	——	Accepted by fowl. Near *P. suanus* Druce, and matches unidentified specimens from Volcan in British Museum collection
Idalus hippia Stoll	B	R	———	M	———
I. daga dares Druce	B	R(III)	———	——	Rejected by *Cebus*
Eupseudosoma aberrans Schaus	B	R(III)	———	L	Rejected by *Cebus*
E. aletis Schaus	B	R(III)	———	L	
Robinsonia similis Rothschild	T	III	———	M-H	———
R. deiopea Druce	B	III	Text fig. 18	M-H	Rejected by *Cebus*
R. sanea Druce	B	III	Text fig. 19	M-H	
R. variegata Rothschild	B	III	Rest with striped abdomen exposed. Convergent to small Pericopidae	M-H	
R. flavomarginata Reich.	B	III			
Baritius hampsoni Dognin	B	II	———	——	Rejected by *Cebus*
B. haemorrhoides Schaus	B	II	———	——	
B. superba Schaus	BS	II	Text fig. 2	——	
Pachydota saduca Druce	S	II	No abdominal component. Text fig. 6	——	
P. affinis Rothschild	T	II		——	
P. punctata Rothschild	B	II		——	Rejected by *Cebus*

TABLE 1. LIST OF SPECIES STUDIED, WITH NOTES ON THEIR DISPLAY TYPES, PALATABILITY AND SOUND PRODUCTION

ARCTIIDAE PHAEGOPTERINAE (77 species)	Locality	Display type	Additional components, etc.	Sound production	Palatability and notes
Melese laodamia Druce	BT	R	Text fig. 16	L	*M. laodamia* rejected by Silver-beaked Tanagers. *M. laodamia, M. asana* and *M. incerta* accepted by both primates. *M. russata* and *chiriquensis* (1 ♂) accepted by fowl on majority of occasions
M. russata H. Edwards	S	R	—	L	
M. asana Druce	B	R	Forelegs sometimes quivered, as in *Bertholdia.* Text fig. 15	L	
M. incerta Walker	BT	R		L	
M. chiriquensis Schaus	S	R	—	L	
Bertholdia grisescens Rothschild +	S	II	Abdomen not curled forelegs raised and quivered. Text fig. 5	L	Accepted by fowl
B. myosticta Hampson	S	II		L	Rejected by fowl
B. fumida Schaus	S	II		L	Single specimen
B. albipunctata Schaus	B	II		L	Single specimen
Neritos cotes Druce	B	R	—	M	Accepted by primates
N. suffusa Rothschild	BT	R	—	M'	
Ochrodota pronapides Druce	BS	R	Text fig. 17	L	Accepted by *Cebus* & fowl
Phaemolis lepida Schaus	B	R	—	L	Accepted by *Cebus*
Neaxia bella Schaus	B	R	—	L	
Eriostepta beata Dognin	B	R	—	L	
Araeomolis persimilis Rothschild	B	R	—	L	
Amaxia pyga Schaus	B	R	—	H	
Amaxia chaon Druce	B	R	—	L	—
Ammalo helops Cramer	B	R	—	—	Rejected by *Cebus*
Elysius conspersa Walker	B	R	—	—	
Amastus rumina Druce	S	R	—	—	—
Opharus astur Cramer	BS	R	—	—	—
Psychophasma erosa Herrich-Schaffer	B	R	—	—	—
Castrica phalaenoides Drury	B	R	—	M	—
Tricypha imperialis Heylaerts	B	R	—	—	Rejected by *Cebus*
Pelochyta misera Schaus	B	R	—	—	—
Halysidota interlineata Walker	B	R	Forelegs raised and quivered as in *Bertholdia.* Abdomen not curled. The display of *H. interlineata* infrequent and transient	—	Rejected by *Cebus*
H. underwoodi Rothschild	S	R-II		—	—
H. atra Druce	S	R-II		—	—
H. cyclozonata Hampshire	B	R	—	L	
H. iridescens Schaus	B	R	—	L	Accepted by *Cebus*
H. marionensis Schaus	B	R	—	—	
H. angulata Rothschild	B	R	—	—	
Glaucostola guttipalpis Walker	B	R	—	M	
G. reimona Schaus +	B	R	—	M	—
G. flavida Schaus	B	R	—	M	Rejected by *Cebus*
G. metaxantha Schaus +	B	R	—	?	One male, July, 1963
Agoraea semivitrea Rothschild	B	II	—	—	—
Metaxanthia aureiventris Rothschild	B	R	—	—	—
M. atribasis Rothschild +	B	R	—	—	Agrees with Forbes's description of specimens from Barro Colorado, but not with authenticated specimens. Possibly a new species.
BELEMNIIAE (3 species)					
Belemnia pavonia Forbes	B	RW	—	—	Rejected by *Cebus*
Belemniastis troetschi Druce	B	RW	Frothing from thorax	—	
Diospage chrysobasis Hampson	B	RW	" "	—	—

TABLE 1. LIST OF SPECIES STUDIED, WITH NOTES ON THEIR DISPLAY TYPES, PALATABILITY AND SOUND PRODUCTION (CONTINUED)

ARCTIIDAE ARCTIINAE (5 species)	Locality	Display type	Additional components, etc.	Sound production	Palatability and notes
Ecpantheria laeta Walker	B	II	—	—	Rejected by *Cebus*
E. muzina Oberthür	T	II	Text fig. 3	—	
E. icasia trinitatis Rothschild	B	II	—	—	Rejected by *Cebus*
E. orsa Cramer	B	II	—	—	
Virbia rosenbergi Rothschild	B	II,W	Some slow elevation and depression of the wings	—	Accepted by *Cebus*
CTENUCHIDAE					
EUCHROMIINAE (35 species)					
Histaea cepheus Cramer	T	RW	Copious reflex bleeding	VH	Sounds made by single male only
Homoeqcera stictosoma Druce	B	RW	Male with ventral valve secretion	—	Rejected by *Cebus*
Autochloris jansonis Butler	B	RW	Frothing of pericopid type	—	Rejected by *Cebus*
Sarosa innotata Draudt	B	RW	"Bobbing" of abdomen	—	Rejected by *Cebus*
Gymnelia salvini Butler	B	RW	—	—	” ” ”
Phoenicoprocta paucipuncta Dyar	B	RW	—	—	” ” ”
P. vacillans Walker	T	RW	—	—	
P. insperata Walker	B	RW	—	—	
Mesothen pyrrha Schaus	B	RW	—	—	
Cosmosoma teuthras Walker	B	RW	—	—	Rejected by *Cebus*
C. batesi Butler	B	RW	—		—
C. hercyna hercynacula Dyar	B	RW	—	—	
C. metallescens Menetries	B	RW	—	—	
C. remotum Walker	B	RW	—	—	
C. advena Druce	S	RW	—	—	
C. klagesi Rothschild	T	RW	—	—	
C. metathoracica Kaye	T	RW	—	—	
C. bogotensis Feld	B	RW	—	—	One male only
Saurita clusia Druce	BT	RW	—	—	Rejected by *Cebus*
S. afflicta Walker	BT	RW	—	—	” ” ”
S. anselma Schaus	B	RW	—	—	” ” ”
Isanthrene crabroniformis Staudinger	S	II	Very wasp-like in flight. Text fig. 4	—	One female only
Psoloptera thoracica Walker	B	RW	—	—	Rejected by *Cebus*
Rhynchopyga cryptoleuca Walker	B	RW	—	—	” ” ”
Dycladia vitrina Rothschild	B	R	—	—	
Phoenarete diana Druce	S	II-W	Display posture assymetrical	—	Rejected by chickens
Macrocneme adonis Druce	B	RW	Male genitalia extruded. Text fig. 1a	—	All *Macrocneme* tested with *Cebus* were rejected
M. thyra Moschler	BT	RW		—	
M. cyllarus Druce	B	RW	—	—	
M. hampsoni Schrottky	B	RW	—	—	
M. auripes Walker	B	RW	—	—	
M. chrysotarsia Hampson	B	RW	—	—	
Calonotos tiburtus Cramer	B	RW	Male genitalia extruded. Text fig. 1a	—	Rejected by *Cebus*
C. metallicus Druce	B	RW		—	” ” ”
C. craneae Fleming	T	TW		—	—

TABLE 1. LIST OF SPECIES STUDIED, WITH NOTES ON THEIR DISPLAY TYPES, PALATABILITY AND SOUND PRODUCTION (CONTINUED)

CTENUCHIDAE CTENUCHINAE (63 species)	Locality	Display type	Additional components, etc.	Sound production	Palatability -and notes
Marecidia achrysa Forbes -I	B	RW	——	——	Rejected by *Cebus*
Eumenogaster moza Druce	B	RW	——	——	" " "
Chrysostola fulvisphex Druce	B	RW	——	——	
Pterygopterus leucomela Walker	B	RW	——	——	Mimics general facies of sphecid wasp. Antennae palpate ground when walking, and movement jerky. Rejected by *Cebus*
Androcharta meones Stollinger	B	RW	——	——	——
Amycles anthracina Walker	B	RW	——	——	
Atyphopsis modesta Butler	B	R	——	——	Certain of these very similar species producesounds when they are handled roughly (M), but the distribution of sound production has not been studied. All are superficially much alike, and all those tested were rejected by *Cebus*. Single specimens from Barro Colorado, tentatively assigned to *E. vinasia* Schaus, *E. klagesi* Rothschild and *E. demonis* Druce have also been taken.
Episcepsis venata Butler	B	R	——	——	
E. lamia Butler	B	R	——	——	
E. lenaeus Cramer	B	R	——	——	
E. hypoleuca Hampson	B	R	——	——	
E. frances Dyar	B	R	——	——	
E. gnomoides Schaus	B	R	——	——	
E. capysca Schaus	B	R	——	——	
E. pseudothetis Fleming	T	R	——	——	
E. redunda Schaus	T	R	——	——	
Eucereon aoris Moschler	B	R	——	L	All species of *Eucereon* tested with *Cebus* or with domestic fowl were rejected. They included *E. obscurum, E. latefascia, E. pseudarchias, E. zamorae, E. scyton, E. decora, E. aeolum, E. tarona, E. leria, E. atrigutta,* and *E. flavicaput*
E. aeolum Hampson	B	R	——	L	
E. obscurum Moschler	B	R	Specialized resting posture. Text fig. 14	L	
E. leria Druce	S	R	Crimson cervical hairtufts. Text figs. 20, 21	M	
E. tarona Hampson	B	R		M	
E. formosa Dognin	S	R	Text fig. 27	M	
E. atrigutta Druce	B,S	R	——	M	
E. venosa Schaus	B	R	——	M	
E. rosa Walker	B	R	——	H	
E. maia Druce	B,T	R	Text fig. 29	H	
E. zamorae Dognin	B	R	——	——	
E. flavicaput Hampson	B,S	R	Text fig. 24	——	
E. scyton Cramer	B	R	——	——	
E. decora Schaus	S	R	Dorsal abdominal hair tufts	——	
E. xanthura Schaus	B	R	——	——	
E. intranotata	B	R	——	——	
E. myrtusa Druce	S	R	——	——	
E. latefascia Walker	B	R	Text fig. 22	——	
E. pseudarchias Hampson	B	R	——	——	
E. striata Druce	S	R	——	——	
E. rogersi Druce	B,S	R	Text fig. 25	——	
E. tigrata Herrich-Schaffer	S	R	Text fig. 26	——	
E. costulata Herrich-Schaffer	S	R	Text fig. 28	——	
E. zephyrus Schaus	B	R	——	——	
Heliura balia Hampson	B	R	Text fig. 23	——	
H. rhodophila Walker -I	B	R	——	——	

TABLE 1. LIST OF SPECIES STUDIED, WITH NOTES ON THEIR DISPLAY TYPES, PALATABILITY AND SOUND PRODUCTION (CONTINUED)

CTENUCHIDAE CTENUCHINAE (63 species)	Locality	Display type	Additional components, etc.	Sound production	Palatability and notes
H. banoca Forbes ‡	B	R	———	——	All *Heliura* and *Delphyre* rejected by *Cebus*
Delphyre rubricincta Hampson	B	R	Foul odor released when handled	——	
D. atava Druce	B	R	Resembles *Episcepsis*	ML	
D. aurorina Forbes ‡	B	R	———	——	
Correbia lycoides Walker	BT	R	Very heavily sclerotized	——	All are lycid beetle mimics, and all are rejected by *Cebus*
C. affinis Druce	B	R	———	——	
Correbidia terminalis Walker	BT	R	Weakly sclerotized	——	
Hyaleucera luctuosa Moschler	B	R-II	———	——	———
H. fusiformis Walker	B	R-II	———	——	
H. gigantea Schaus	BS	R	Resting posture and behavior like *Eucereon obscurum*	——	
Ptychotrichos zeus Schaus	B	II	Abdomen curled, wings not elevated	——	
Napata walkeri Druce	BT	R	All are tough and bear bold patterns when they are at rest	L	All are rejected by *Cebus*
N. leucotelus Butler	B	R		L	
Aclytia gynamorpha Hampson	B	R		L	
A. punctata Butler	B	R		L	
A. albistriga Schaus ‡	B	R		L	
Leucopleura ciarana Schaus ‡	B	RW	Genitalia extruded by both sexes	——	Rejected by *Cebus*
Xanthopleura troetschi Druce	B	RW	———	——	———

stimulus. It may be associated with other, more specialized, postures, for example, the extrusion of colored genital processes, of colored tubes from the cervical region, or of abdominal hair tufts (see below). This is the typical protective response of the ctenuchids, and two examples are given in Text-fig. 1.

III. *Wing-waving Displays.*—The wings, which are protracted from rest, are slowly and gently elevated and depressed. These displays are similar to the saturniid rhythmic displays (Types III & IV) (Blest, 1957). They are found in the North American *Diacrisia virgo*, in virtually all the mimetic Ctenuchidae as a concomitant of walking, and in *Virbia* spp, following Type II display. Transitional genera between the Arctiidae and Ctenuchidae (*e.g., Belemnia, Belemniastis* and *Diospage*, but not *Metaxanthia*) also wave their wings as they walk.

IV. *Type II Sustained Static Displays.*—These have been described for the hemileucine Saturniidae (Blest, 1957). The moth falls from its substrate, curls the abdomen so that the dorsal surface is convex, and places its legs in a characteristic posture; the wings are simultaneously elevated (Text-figs. 2-6). Static displays of this kind may be maintained for periods of a few seconds up to several minutes by saturniids such as *Dirphia (Periphoba) hircia* (Blest, 1960). Among the present families these displays show more variety: the abdomen is not always curled, and the angle of wing elevation and leg-extension varies widely between species.

V. *Type III Sustained Static Displays.*—Displays of this type are the converse of the Type II displays; the abdomen is elevated instead of depressed, so that it projects above the folded wings at right angles to the substrate, and the wings are slightly depressed. The abdomen itself is usually bright colored and banded (Text-figs. 7-12).

VI. *Special Devices:*

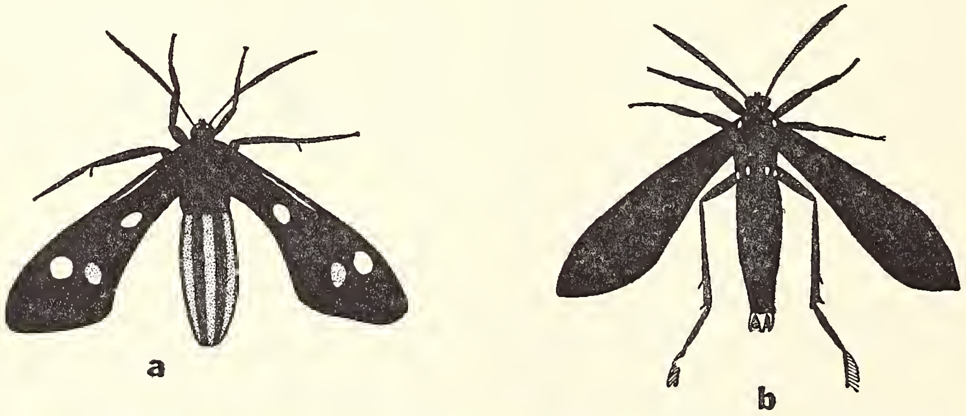
(i) *Extrusion of the Male Genitalia.*—The male genital processes may be ornamented with brightly-colored tufts of hair (*e.g., Baritius haemorrhoides*), or they may be so feebly sclerotized that they assume the color of the blood (*e.g., Macrocneme thyra intacta, Calonotos craneae*). In species so decorated the genitalia are extruded during display (Text-fig. 1).

(ii) *The Display of Abdominal or Cervical*

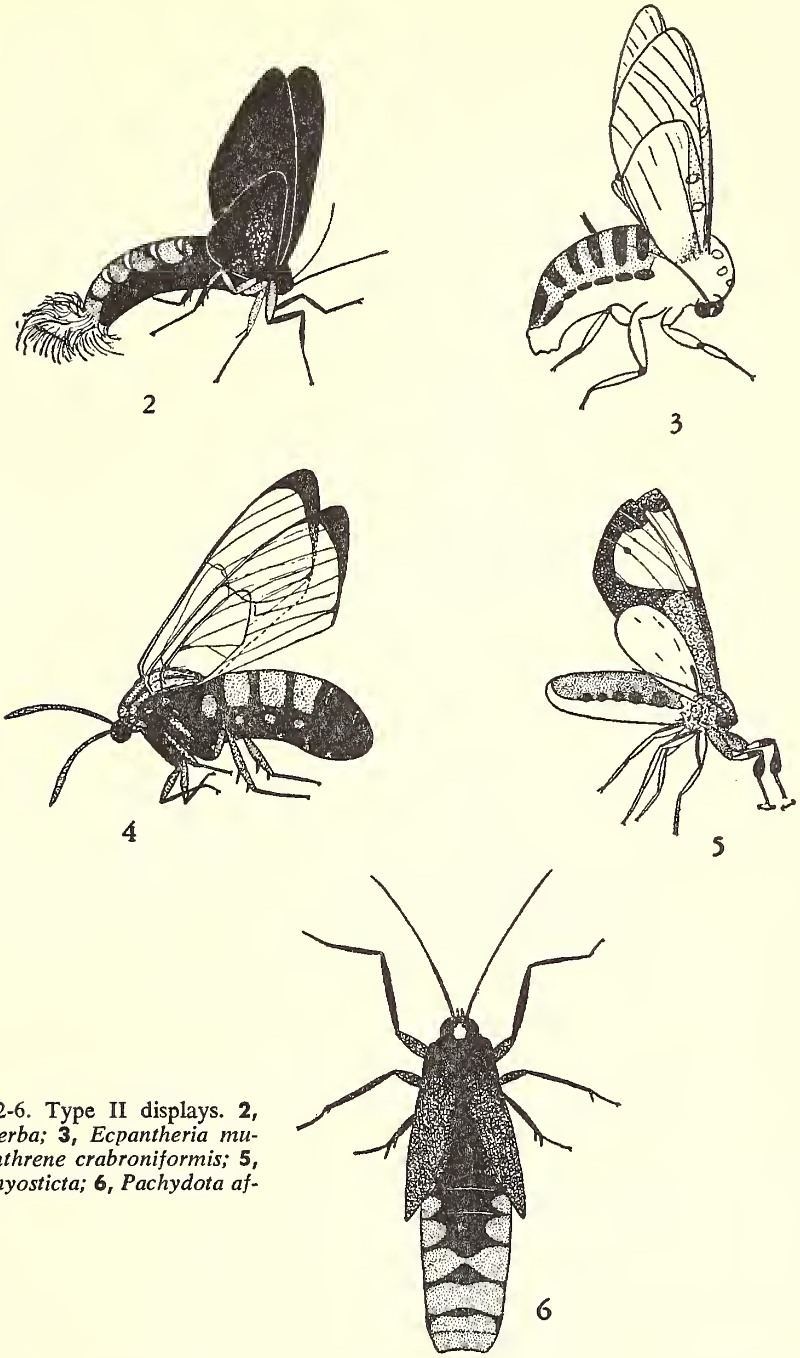
TABLE 2. FREQUENCY WITH WHICH MALE MOTHS WERE CAPTURED AT HOURLY INTERVALS THROUGHOUT THE NIGHT OVER A THREE-WEEKS PERIOD (JUNE 30–JULY 25, 1962), ON BARRO COLORADO ISLAND

I. ARCTIIDAE Flight Times Species	19:00-20:00	20:00-21:00	21:00-22:00	22:00-23:00	23:00-24:00	24:00-01:00	01:00-02:00	02:00-03:00	03:00-04:00	04:00-05:00	Totals	Sound Threshold
Melese laodamia	3	1	1	0	1	3	0	1	118	19	147	L
M. incerta	1	2	2	3	1	4	8	33	60	20	134	L
M. asana	1	0	1	0	3	0	0	0	2	2	9	L
All *Melese*	5	3	4	3	5	7	8	34	180	41		
Automolis obscurata	1	3	0	0	1	0	0	1	4	13	22	L
Halysidota iridescens	0	1	0	0	0	0	1	5	15	7	29	L
Automolis dilutus	1	1	17	20	4	2	0	0	1	1	47	H
A. critheis	0	3	7	5	0	1	0	0	0	0	16	Silent
A. rutila/A. chionoplaga	2	3	0	0	0	0	0	1	0	0	6	H
A. elissa/A. elissoides	0	0	0	0	0	0	3	0	0	0	3	H
A. sicilia/A. metallica	1	1	1	0	3	2	2	0	0	0	10	H
A. lineosa	0	0	1	4	2	0	1	0	1	0	9	L-M
All *Automolis*	4	8	26	29	9	5	6	1	2	1		
Robinsonia variegata/ R. sanea/R. deiopea	1	0	1	2	2	0	2	0	0	1	9	M-H
Amaxia pyga	1	0	0	2	2	10	5	3	2	0	24	H
Elysius conspersa	0	0	0	3	10	5	2	1	0	0	21	Silent
II. CTENUCHIDAE												
Eucereon pseudarchas	0	3	3	6	4	3	3	0	4	0	26	Silent
Aclytia gynamorpha	3	9	6	2	0	0	2	0	0	2	24	L
Episcepsis spp.	3	13	6	6	3	2	3	0	2	2	40	M-silen
Correbidia terminalis	8	24	15	8	5	1	0	1	0	0	62	Silent
Correbia lycoides	7	11	5	0	1	2	0	0	0	0	26	Silent
Delphyre rubricincta	11	8	2	5	0	0	0	0	1	0	27	Silent
Calonotos tiburtus	6	31	3	0	1	0	0	0	0	0	41	Silent
C. metallicus	2	8	1	2	0	0	0	0	0	0	13	Silent
Macrocneme spp.	23	22	2	2	0	1	1	0	0	0	51	Silent
Homoeocera stictosoma	0	0	1	7	2	4	3	1	0	0	18	Silent

Hair Tufts.—Some ctenuchids (*e.g., Eucereon decora*) have a series of lateral hair tufts on the anterior part of the abdomen, which is concealed at rest, and only erected during display. Display is of Type II, and the erected hair tufts are therefore readily visible to a predator. Some



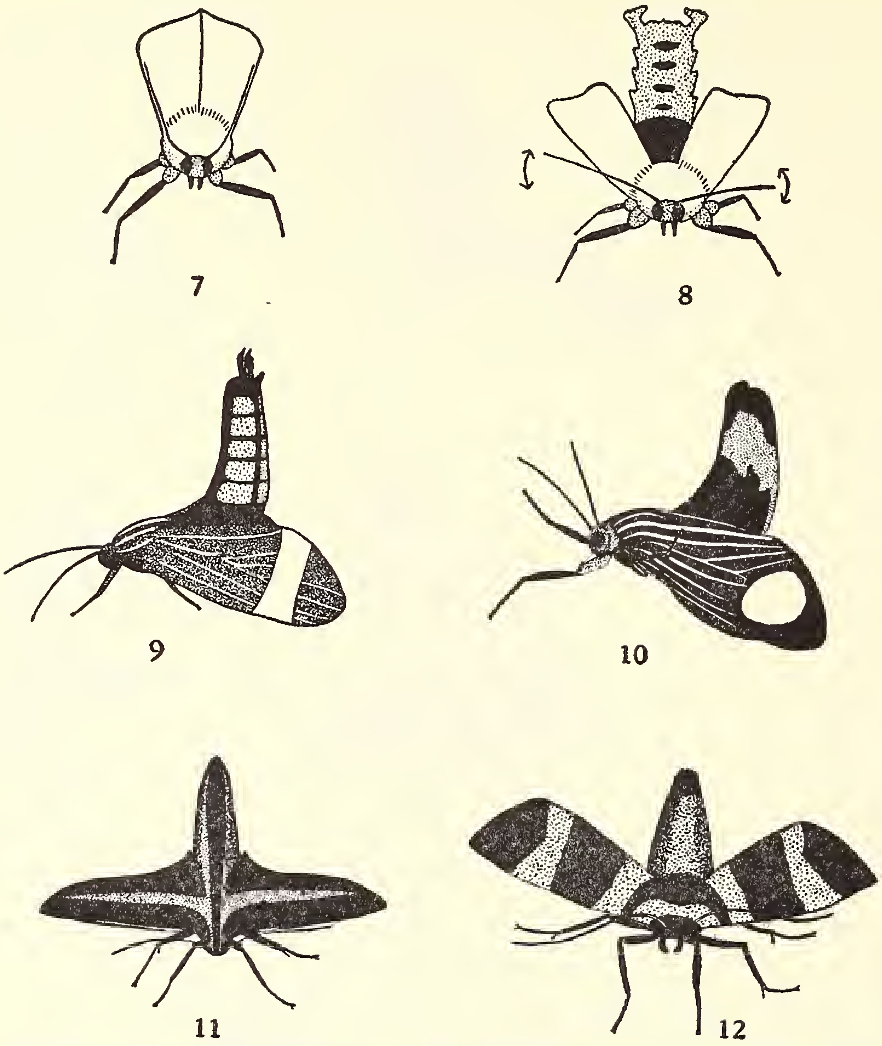
TEXT-FIG. 1. **a**. *Calonotos craneae,* reflex immobilization, female. **b**. *Macrocneme thyra intacta,* male. Reflex immobilization, with slight extrusion of the genitalia.



Text-figs. 2-6. Type II displays. **2,** *Baritius superba;* **3,** *Ecpantheria muzina;* **4,** *Isanthrene crabroniformis;* **5,** *Bertholdia myosticta;* **6,** *Pachydota affinis.*

Eucereon spp. and related genera have red or orange cervical hair tufts which are only exposed when the moths are handled.

(iii) *Release of Materials from the Ventral Valve.*—The males of certain ctenuchids possess a specialized ventral abdominal structure, the ventral valve, which in some species encloses a yellow or whitish secreted product. In only one of the species observed in this study has any directly protective function been noted for this material, though it may assist in rendering the moths unpalatable. In *Homeocera stictosoma,* light restraint of the insect elicits reflex immobilization, and the instant release of the accumu-



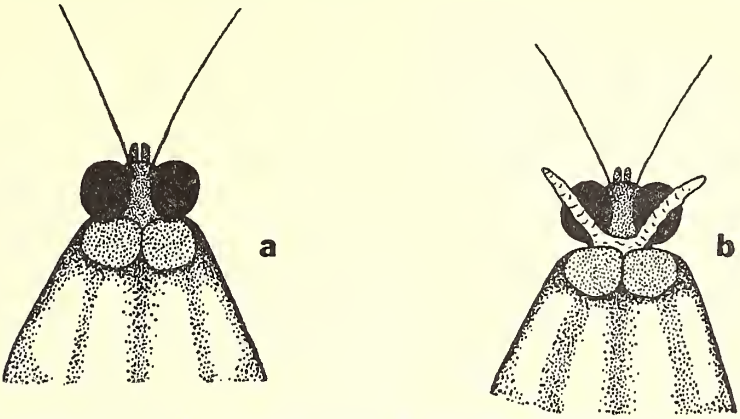
TEXT-FIGS. 7-12. Type III displays. **7,** *Automolis elissoides* at rest; **8,** *A. elissoides* displaying; **9,** *A. rutila;* **10,** *A. chionoplaga;* **11,** *A. taeniata;* **12,** *A. tegyra.*

lated secretion, in the form of a light white flocculent material composed of fine friable fibres which rapidly envelop the animal, and which have a faint but definite phenolic odor. They are dispersed when the insect flies away. The material is not replenished after it has been used, and the ventral valves of animals which have released it remain empty until they die.

(iv) *Reflex Bleeding.*—In a great many species reflex immobilization is accompanied by reflex bleeding from the joints and prothorax. It is particularly copious in *Histaea cepheus* and *Amastus rumina.*

(v) *Specialized Secretions.* — Several European arctiids are known to possess specialized defensive secretions which are emitted from glands in the prothorax (Bisset, Frazer, Rothschild & Schachter, 1960). In one case *(Arctia caja* (L.)), the secretions have been shown to contain an unusual higher alkyl choline ester, probably β-β-dimethylacrylylcholine, or a near derivative (Bisset *et al., loc. cit.*). It is likely that some of the species under consideration may possess such defensive mechanisms, but they were not specifically searched for in this study.

(vi) *Odors.*—Most arctiids and ctenuchids which are definitely distasteful have a noticeably unpleasant, musty odor to the human observer, and they are often rejected by *Cebus* in test situations after being smelled but not tasted. These odors are absent or less noticeable in



TEXT-FIG. 13. **a.** & **b.** Cervical region of unidentified ctenuchine near to *Eucereon,* to show extrusible lemon-yellow processes.

species which have proved palatable to both *Cebus* and *Oedipomidas (e.g., Melese laodamia* and *M. asana).* Odor is not, however, a necessary concomitant of unpalatibility, for *Bertholdia myosticta,* which were consistently rejected by chickens, do not possess a strong smell.

Delphyre rubricincta, which performs a Type II display exhibiting a black and crimson banded abdomen, simultaneously releases a foul odor of short persistence. No visible droplets of fluid have been seen to accompany it, and its origin is consequently for the present unknown. This odor somewhat resembles that of the alarm substance of the large neotropical ants of the genus *Paraponera.* Specimens of *Gymnelia salvini* have a faint but similar odor.

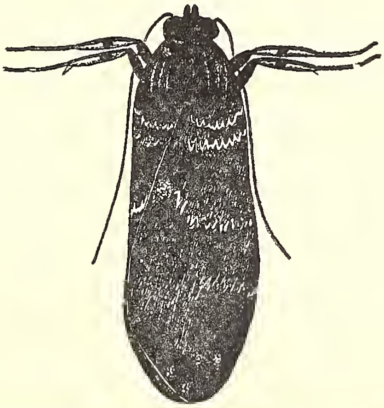
(vii) *Frothing. — Belemniastis troetschi* and *Autochloris jansonis* froth vigorously from the thorax when they are handled. Many pericopids produce froth and so do African members of the genus *Rhodogastria* (Arctiidae) (Carpenter, 1938).

(viii) *Other Specialized Components.*—Although the general forms of the displays are remarkably stereotyped, the minor components are often modified. For example, species of *Baritius* tend to retain their hold on the substrate while performing a Type II display, and *B. haemorrhoides* and *B. superba* back away from the disturbing stimulus, following a semicircular path. *Bertholdia* spp. place the first pair of legs in a specialized posture and quiver them rapidly (Fig. 5); similar specialized leg postures are also found in *Melese asana* and *M. incerta,* and in *Halysidota underwoodi.*

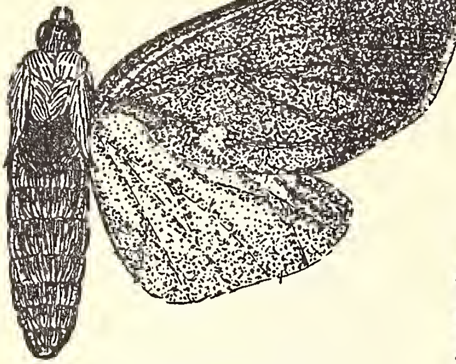
The ctenuchid *Sarosa innotata* accompanies an otherwise normal reflex immobilization with vertical bobbing movements of the abdomen, which is yellow with a black tip; the significance of this behavior pattern is obscure, and similarly marked species of *Cosmosoma (e.g. C. hercyna hercynacula)* do not possess it.

A single specimen of an unidentified ctenuchine near *Eucereon* from Santa Clara extruded a pair of flabby lemon-yellow processes from the cervical region when touched (Text-fig. 13). No perceptible odor was associated with them. *E. leria* and *E. tarona* bent their heads forward to expose crimson hair tufts in the same region.

Perhaps the majority of ctenuchids show some degree of mimicry, hymenopterous and dipterous insects being the most usual models. A general account is given in the various sections of Seitz's "Macrolepidoptera of the World" dealing with the family. Although the flight patterns of these mimetic species often closely resemble those of the various models, their resting behavior is relatively little specialized; the more extreme wasp-mimics, however, *(e.g., Pterygopterus leucomela)* walk with the jerky movements of their models, and may even palpate the surface of the ground with their antennae in a wasp-



TEXT-FIG. 14. The resting posture of *Eucereon obscurum.*



Text-fig. 15

Text-fig. 16

Text-fig. 17

Text figs. 15-17. Cryptic and procryptic species. **15,** *Melese incerta;* **16,** *M. laodamia;* **17,** *Ochrodota pronapides.*

like manner. An account of some ctenuchid flight adaptations is given by Beebe & Kenedy (1957).

VII. *Cryptic Coloration.*—Although several genera have an inconspicuous appearance, perhaps equivalent to "generalized cryptic coloration" in the saturniids (Blest, 1957) *(e.g., Melese, Bertholdia, Elysius, Ammalo)* (Text.-figs. 15 and 16), very few appear to have specialized procryptic coloration. The exceptions, which have been shown to be palatable *(Ochrodota pronapides* (Text-fig. 17), *Amaxia pyga, Melese chiriquensis),* are also striking for their fragility; this correlation between procrypsis and fragility can probably be explained in terms of natural selection, and requires more extensive study (Blest, 1963a).

4. Notes on Individual Genera

Although a list of the species and their behavior is given in Table I, certain genera deserve special discussion.

I. *Automolis.*

Forbes (1939) considers that this genus is not homogeneous, and it has been split into a number of species-groups. Its protective behavior is of two kinds: reflex immobilization, and Type III display. Either may be accompanied by sound production, the threshold for which is lowest in those species showing reflex immobilization only, and highest in *A. taeniata,* only one of some two dozen specimens producing an auditory signal. Since *A. taeniata* maintains its display particularly vigorously, it would appear that this relation may be merely a matter of the occlusion of sound production by the competing display reflex.

Despite the uniformity of the Type III displays, the visual patterns which they exhibit vary widely (Text-figs. 7-12). *A. dilutus* apparently mimics an implausibly large lampyrid beetle and does not display. Tactile or visual stimuli cause it to fall to the ground, and the related *A. reducta* behaves in the same way.

All members of the genus tested showed some degree of unpalatability; some species *(e.g., A. sicilia, A. vitrea)* were occasionally accepted by *Cebus,* although the majority of individuals were rejected. Most species were consistently rejected *(A. metallica, A. diluta, A. critheis, A. elissa, A. elissoides),* and survived investigation and rejection by *Cebus* on the majority of occasions. *A. taeniata* proved highly distasteful to domestic fowl, and individual birds rapidly learned to ignore them.

II. *Cissura.*

This genus was represented by one species only, *C. plumbeus* on Barro Colorado. It is notable for the coupling of reflex immobilization to sound production at very high threshold, the signals consisting of a brief train of short squeaks. The coloration is aposematic, and comprises longitudinal carmine stripes on a dark slate-gray ground. This was the only species which all the *Cebus* in the colony learned to ignore on sight, and of which every individual survived investigation during the early encounters.

III. *Robinsonia.*

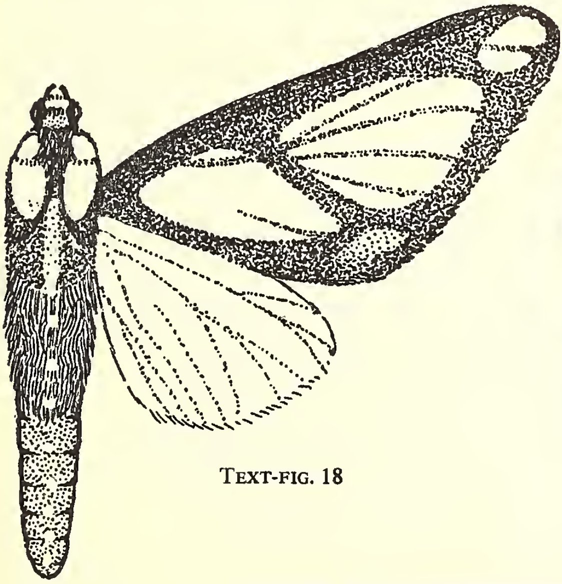
All members of this genus have Type III displays, and all produce sounds. Two of the species *(R. deiopea* and *R. sanea),* (Text-figs. 18 & 19), have uniformly pale yellow abdomens and rest in the normal arctiid position, with the abdomen concealed by the folded wings. *R. flavomarginata* can be found resting on the undersides of the leaves of low second-growth vegetation in the forest; this and the similar *R. variegata* show convergence towards the smaller pericopids which may be found in the same habitat, particularly in the concentration of dark pigmentation along the veins of the forewings; they share the pericopid habit of resting with the brightly colored abdomen exposed.

IV. *Eucereon, Delphyre, Heliura, Hyaleucera gigantea.*

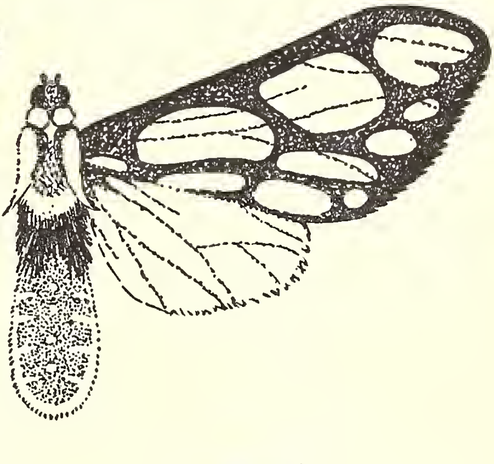
All the species of this group which have been tested have proved unpalatable. The 27 species from Barro Colorado and Santa Clara assort themselves into the following groups on the basis of their wing patterns; 24 belong to *Eucereon,* to which three other genera are convergent.

This grouping omits *Delphyre atava,* which resembles an *Episcepsis,* and *D. aurorina, Heliura banoca* and *H. rhodophila,* which neither resemble each other nor any of the main groups of *Eucereon.* Group II is more diversified than the others, and no account is taken of the numerous species of *Eucereon* found in Trinidad.

Each of these types of pattern is distinct, and the members of each group are closely similar to each other. The species in most cases are adequately illustrated by Seitz. The distribution of these species between Santa Clara and Barro Colorado suggests the following conclusions: (i) In most cases each group can be treated as two small subgroups of similar sympatric species, with little overlap. (ii) Each such subgroup functions as a potential Müllerian assemblage. (iii) There is a rather striking pattern of replacement within each group. Thus, in Group I,



TEXT-FIG. 18



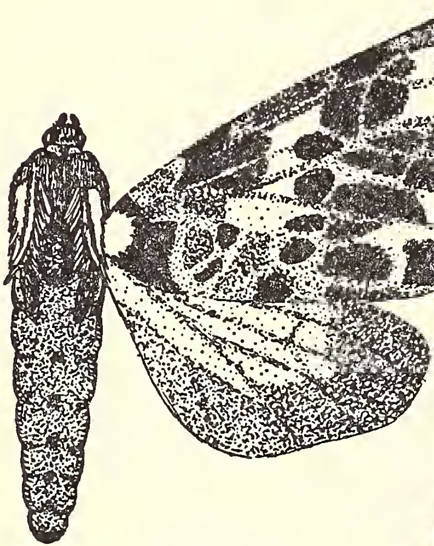
TEXT-FIG. 19

TEXT-FIGS. 18 & 19. *Robinsonia.* **18,** *R. deiopea;* **19,** *R. sanea.*

Group I		Group II		Group III		Group IV	
leria	(S)	*aeolum*	(B)	*zamorae*	(B)	*atrigutta*	(BS)
tarona	(B)	*aoris*	(B)	*flavicaput*	(BS)	*formosa*	(S)
		pseudarchias	(B)	*scyton*	(B)	*tigrata*	(S)
(Text-figs. 20, 21).		*latefascia*	(B)	*decora*	(S)		
		myrtusa	(S)	*xanthura*	(B)	(Text-figs. 26, 27).	
		rosa	(B)	*rogersi*	(S)		
		Heliura balia	(B)				
		Delphyre rubricincta	(B)	(Text-figs. 24, 25).			
		(Text-figs. 22, 23).					
Group V		**Group VI**		**Group VII**		**Group VIII**	
striata	(S)	*obscurum*	(BS)	*zephyrum*	(B)	*maia*	(B)
costulata	(S)	*Hyaleucera gigantea*	(BS)				
phaeoprocta	(B)					(Text-fig. 29)	
intranotata	(B)	(Text-fig. 14).					
(Text-fig. 28).							

B = Barro Colorado; S = Santa Clara.

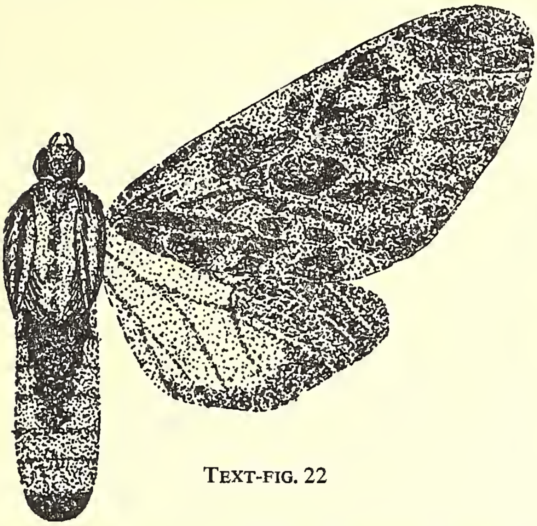
tarona on Barro Colorado is replaced in Santa Clara by the very closely similar *leria;* in Group V, *phaeoprocta* and *intranotata* by *costulata* and *striata*. The close similarity between the patterns and coloring found in the two habitats in these distasteful species is in striking contrast to those of the predominantly procryptic families such as the Lasiocampidae and Noctuidae, where the wealth of lichens, mosses and epiphytes at the higher altitude has provoked the evolution of patterns and coloration distinct from the forms prevalent on Barro Colorado (Blest, 1963a).



TEXT-FIG. 20

TEXT FIGS. 20 & 21. *Eucereon*, Group I. **20,** *E. tarona;* **21,** *E. leria.*

TEXT-FIG. 21



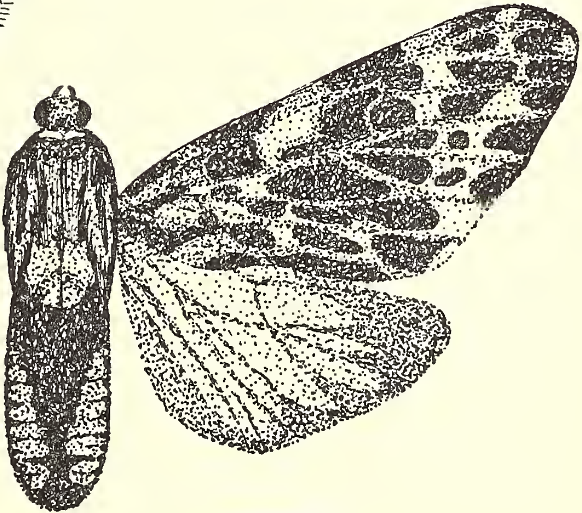
TEXT-FIG. 22

TEXT-FIG. 23

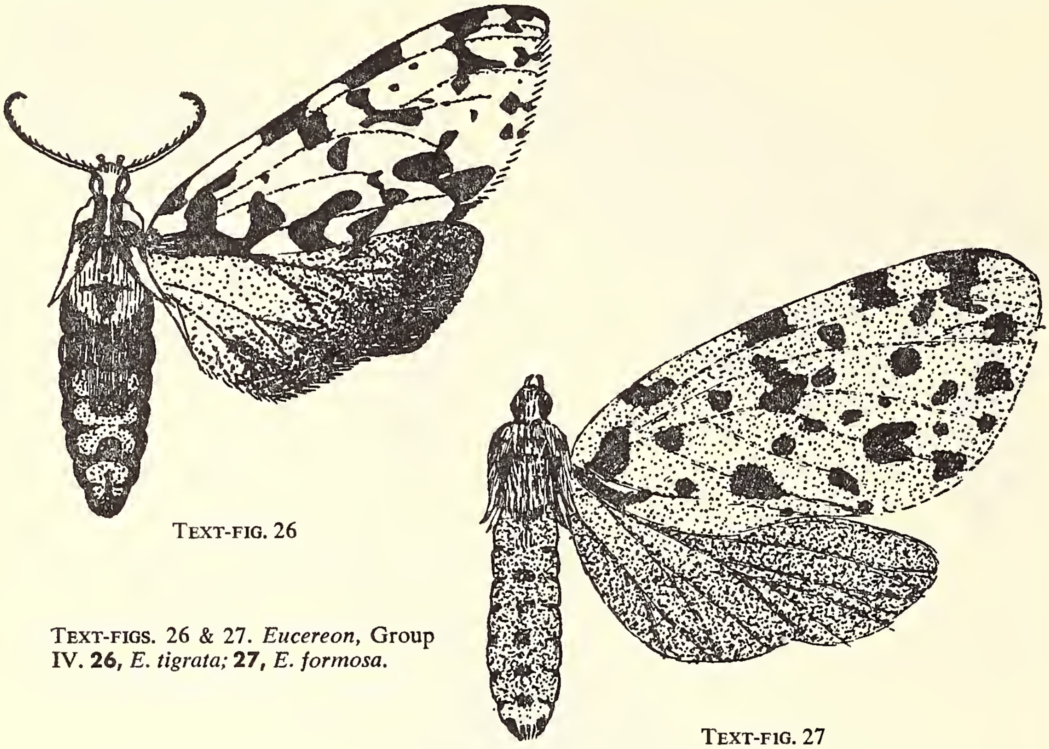
TEXT-FIGS. 22 & 23. *Eucereon,* Group II. **22,** *E. latefascia;* **23,** *Heliura balia.*

TEXT-FIG. 24

TEXT FIGS. 24 & 25. *Eucereon,* Group III. **24,** *E. flavicaput;* **25,** *E. rogersi.*



TEXT-FIG. 25



Text-fig. 26

Text-figs. 26 & 27. *Eucereon,* Group IV. **26,** *E. tigrata;* **27,** *E. formosa.*

Text-fig. 27

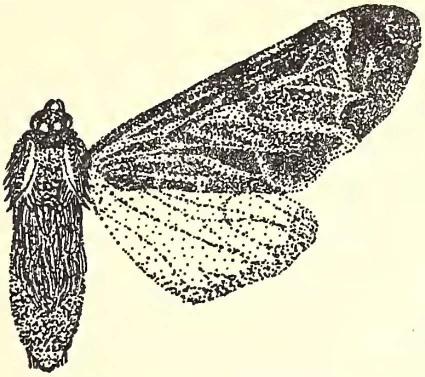
It is possible that the existence of similar groups of "equivalent" species in the two areas is merely a function of the niches available to the larvae, and of the stability of the color patterns within the groups. However, there still remains the possibility that the distribution of aposematic patterns within populations of sympatric species is determined by some feature of predator behavior which has not yet been appreciated. For example, Van der Vecht (1961) has provided some support for regarding the action of local predator populations as selectively critical, in a study of the Indo-Australian wasps of the genera *Eumenes, Pareumenes* and *Pseudeumenes.* In this case, *Eumenes flavopictus,* extending from southern India to New Guinea, is mimicked by species of *Pseudeumenes* and *Pareumenes.* All three genera show parallel variation throughout their range, and form in each locality where they simultaneously occur a distinctively patterned Müllerian assemblage. The size of the departure from the pattern of the mainland *Eumenes* population relates directly to the degree of isolation of the divergent populations. It seems necessary to assume that in each isolate specific local patterns of predation have stabilized the dominant species *(Eumenes flavopictus)* and have caused convergence with the other two genera. This interpretation holds whether or not genetic drift is invoked to explain the initial divergence between the island and the mainland populations.

V. *Macrocneme, Calonotos.*

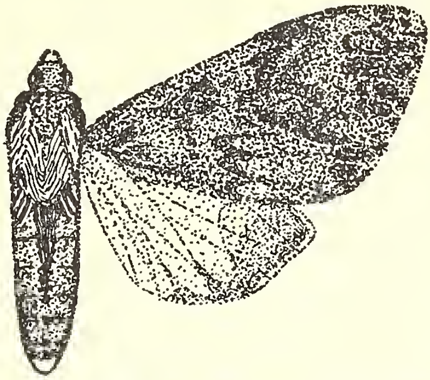
In both these ctenuchid genera, the male genital apparatus has been elaborated in certain species to provide a warning signal. *Macrocneme thyra intacta* and *M. adonis* belong to a division of the genus in which the male ventral valve is large and bifid. The whole genital apparatus, which is bright yellow in life, is extruded during reflex immobilization. In the remaining species it remains retracted, and the valves are small and usually asymmetrical (Forbes, 1939). A similar trend is found in *Calonotos.* Males of *C. craneae* possess supernumerary genital processes and instantly extrude the genital apparatus when restrained. It is feebly sclerotized, bright yellow, and has a fleshy appearance in life. *C. tiburtus* and *C. metallica* lack the extra process; the apparatus is normally sclerotized, brown, and is not spontaneously extruded during handling.

5. Systematic Considerations

Little can be concluded from the distribution



TEXT-FIG. 28. *Eucereon,* Group V. *E. costulata.*



TEXT-FIG. 29. *Eucereon,* Group VIII. *E. maia.*

of display behavior within the two families. Within the Arctiidae, it can be said that no arctiine possesses a Type III display, which is only found in certain Phaegopterinae *(Automolis, Robinsonia* spp.). Type II displays are found in both subfamilies, and in certain ctenuchids. In general, the imprecise morphological division between the ctenuchids and arctiids, most evident in such transitional genera as *Belemnia, Belemniastis* and *Diospyge,* is paralleled by their behavior. Such ctenuchines as *Delphyre* and *Eucereon* spp. differ little in their behavior (reflex immobilization or Type II static displays) from arctiids such as *Agoraea* and *Ecpantheria* spp. The abandonment of the characteristic arctiid displays and resting and reflex immobilization postures follows the adoption by the ctenuchids of varying degrees of mimicry of non-lepidopterous insects, and reduced wing-scaling.

6. RELATIONS BETWEEN ENVIRONMENTAL TEMPERATURE AND DISPLAY BEHAVIOR

It is already clear that the persistence of static displays shows some relationship to environmental temperature. The ambient diurnal temperature on Barro Colorado fluctuates around 27° C at a height of 55 ft. in the forest shade (Allee, 1926), and may reach 34° C during the day. This latter temperature is nearing that of working lepidopterous muscle as measured by Krogh & Zeuthen (1941) for temperate nymphalids and sphingids and by Dorsett (1962) for tropical sphingids. It follows, and observation confirms, that on Barro Colorado only small periods of shivering need precede fully competent flight. Indeed, large and heavy sphingids and saturniids often make successful escape flights without shivering at all. It might be argued, then, that for a species only moderately unpalatable and not physically resilient, selection might well favor escape flights over a protracted display. In fact, this appears to be the case. The static displays of the Barro Colorado arctiids and ctenuchids are transient, and are only held for periods of seconds; so much so that they have proved difficult to photograph.

At Santa Clara, however, at an altitude of 4,000 ft., the temperatures are lower. Apparently in correspondence, displays and reflex immobilization are long-persisting. *Automolis taeniata* and *A. tegyra* maintained Type III displays invariably for periods of seconds, and sometimes for minutes. On Barro Colorado, *A. tegyra,* and *A. metallica* which belongs to the same species-group as *A. taeniata,* both gave only transient displays. At Volcán itself, *Halysidota underwoodi* gave a lengthy display which added specialized leg postures to reflex immobilization. At Barro Colorado, *H. interlineata,* closely related to it, assumed reflex immobilization without leg components for no more than brief periods.

The evidence from arctiid and ctenuchid displays alone does not allow a certain distinction between the effects of natural selection for a display length adjusted to the diurnal ambient temperature, and the mere effect of immediate temperature upon the physiological and behavioral responses of the individual moths. Controlled experiments were not possible, but chilling *Automolis* on Barro Colorado did not seem to lengthen their displays by a substantial amount. The best evidence favoring the first alternative comes from the hemileucine saturniids; there, the palatable forms in general bear eyespot patterns, and either display them statically, or perform rhythmic displays. With the exception of *Automeris janus,* which occurs at a wide range of altitudes, and rare individuals of *A. tridens* and *A. junonia,* none of the Barro Colorado *Automeris* with simple static displays (8 species) perform rhythmic components, while of the Santa Clara *Automeris* which were seen as adults, two *(A. nyctimane* and *A. subpicta)* had low-threshold rhythmic displays, while one *(A. montezuma)* had rhythmic leg components. *A. nyctimane,* received in England as pupae from Bogotá, Colombia (8,000 ft.), also had low-threshold rhythmic displays. All were well sustained, in contrast to the transient displays of the Barro Colorado *Automeris.*

In addition, two species, *A. junonia* and *A. tridens,* perform brief static displays on Barro Colorado, and chilling fails to elicit a rhythmic performance. In the Arima valley of Trinidad (diurnal temperature fluctuating around 25° C) a proportion of individuals of both species perform rhythmic displays, as does the related *A. liberia.* The species whose displays are characteristically of low threshold (those of the *A. nyctimane* group) are typically either montane, or found at the extremes of the range of the genus, in southern Brazil and Argentina, and in Mexico. A detailed study of their distribution in terms of their environmental temperatures and display performance would be of great interest.

7. The General Adaptiveness of Display and Escape Behavior

Probably the most important predators of these small moths are insectivorous birds, notably the numerous species of tropical flycatcher (Tyrannidae) and bats, though nothing is known about the patterns of predation prevailing in the forest canopy. It is likely that birds discriminate more carefully in their choice of prey than the captive monkeys used in these observations, if only for the reason that monkeys caged in groups are in a competitive situation in which prey is eaten to assert dominance, or even to please the experimenter, as well as to satisfy hunger. For example, Silver-beaked Tanagers rejected *Melese laodamia*[3] while the two primates accepted it, and domestic fowl took the equivalent species *(M. russata)* at Santa Clara. For these reasons it is remarkable that so many of the species achieved almost complete protection against the caged *Cebus.* The small number of experimental predators and the erratic appearance of any one species of moth precluded formal palatability tests of the kind so elegantly used by Brower (1958, 1960); tests with *Cebus* were confined to some dozen presentations to the colony in each day, in which no more than eight arctiids were presented, the remaining species being palatable sphingids, noctuids and geometrids. The moths were pushed through the wire, and in most cases the monkeys took them directly in their hands.

From the observations which resulted, it appears that there are two rather distinct ways in which an unpalatable species may survive investigation by an inexperienced monkey. *Cebus* pick up small prey in their hands, and smell it before biting it. Arctiids and ctenuchids are usually rejected at this stage. An actively struggling prey is usually picked to pieces, however, even if it is not subsequently eaten; the rejection tends to be followed by manual investigation which is sufficiently vigorous to elicit struggling from those saturniids which have static displays. Arctiids, however, remain passive throughout this phase, and are ultimately dropped. In nature they would fall some 30-90 ft. from the forest canopy to the ground. Even in cage conditions they succeed in creeping away without exciting further attention. Correlated with this behavior is an outstandingly tough and leathery consistency. Such moths (*e.g., Automolis metallica* and related species) will suffer quite violent investigation without lethal injury.

The alternative method of escape exploits the fact that a monkey when holding a passive prey eventually opens its hands to look at it; species such as *Eucereon* and the euchromiine mimics of Hymenoptera and Diptera terminate their reflex immobilization with instant flight as soon as tactile restraint is removed. *Eucereon aoris* and small *Macrocneme* repeatedly eluded *Cebus* in this way.

It is likely that these two escape mechanisms may succeed against most small primates, and perhaps avian predators as well.

8. Discussion

The main problem raised by these observations may be simply stated: the arctiids and ctenuchids consist of predominantly distasteful insects. So unpalatable are they that most species are rejected even by caged colonies of *Cebus.* If it is assumed that predators in nature must learn to avoid distasteful species, and that those which search visually learn best bold aposematic patterns of a conventional kind (Cott, 1949), then it follows that selection should tend to promote Müllerian convergence in any one taxonomic group of distasteful animals, given certain simplifying conditions in the ecological situation (Blest, 1963a). These conditions consist, in the main, of the mere absence of various sources of constraint. Convergence, for example, might be prevented were the animals to use visual signals for sexual recognition, in which case selection for character divergence as an isolating

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[3] Tests with Silver-beaked Tanagers were conducted by Mr. T. S. Collett and Mr. C. T. Collins in Trinidad.

mechanism (Brown & Wilson, 1956) should tend to sustain the opposite change in any one ecological assembly. Interactions between sexual and mimetic demands of just this character have been described by Stride (1956, 1957). Associations between specialized procryptic coloration and highly evolved habitat-selection could also bring about the same result. In the present case neither of these restraints would appear to operate. The majority of species in both families are highly and efficiently distasteful; they are not, in general, cryptic at rest, and since they are fully nocturnal (with the exception of the Ctenuchinae and some Euchromiinae), there is little likelihood that their courtship involves visual signals. Yet the course of evolution within these families seems often to have involved the maximum divergence of color patterns, even within genera. This is particularly striking in the case of the *Automolis* spp. illustrated in Text-figs. 7-12; all have Type III displays, and some, at least, are unquestionably closely related, (*A. chionoplaga, A. priscilla* and *A. rutila,* for example), yet the overall patterns are very distinct. Of the 16 *Automolis* found on Barro Colorado, only three *(A. elissa, A. elissoides* and *A. salma*) are closely similar to each other. On the other hand, there are apparently convergences across genera (some *Episcepsis, Delphyre atava* and *Aclytia ventralis* are much alike) or even families *(e.g.,* the arctiid *Automolis rutila* and part of the ctenuchine genus *Aclytia),* and even participation in outside mimetic complexes (for example, wasp-mimicry, carried to such extremes as in the ctenuchid genera *Pseudosphex* (Beebe & Kenedy, 1957), and *Pterygopterus,* and the association of *Correbia* and *Correbioides* spp. (Ctenuchidae) with lycid beetles and their mimics; a North American lycid mimetic assembly is discussed in detail by Linsley, Eisner & Klots (1961). Finally, there are those genera which have maintained a close internal similarity; many of the numerous species of *Macrocneme* can only be distinguished reliably by genital characters, and the large distasteful genera *Eucereon* and *Episcepsis* each contains numerous closely similar species.

These discrepancies between simple prediction and fact need explanation; at the least they suggest the hazards of facile evolutionary speculation. Although the confusing and contradictory situation which is presented could be explained on the assumption that much of the adult coloration results from pleiotropic genes whose adaptive significance lies in other spheres, it is preferable to assume that the adult patterning is adaptive. Given this premise, two types of problem are presented:

(i) The case represented by *Automolis,* where intrageneric divergence has been achieved without, in general, any close Müllerian resemblance to co-existent species outside the divergent group.

(ii) The case presented by many ctenuchine and euchromiine species where divergence has implemented Müllerian resemblances to non-lepidopterous insects.

These trends can only be understood in relation to the behavior of predator populations, and the type of information that is needed for their analysis is almost wholly lacking. The selection pressures are perhaps most likely to relate to the distribution of feeding habits within the assemblies of predator species. Research to date has been largely concerned to demonstrate that the learning and discriminatory capacities of single predator species are a sufficient explanation of the mimetic detail which is actually found in nature. It is well known that those highly aposematic and distasteful species which have been studied prove to be the victims of one or more natural predators which are undeterred by devices which are effective against more fastidious enemies (Cott, 1949). For example, whereas highly distasteful hemileucine saturniids are rejected consistently by marmosets, the less fastidious, and, in caged colonies, more highly motivated *Cebus* will take them, and quite soon learn to rub off the distasteful vestiture of hairs and scales (Blest, 1963b, and unpublished data). It has been correctly recognized that the existence of such predators, immune to the protective mechanisms of particular species, does not invalidate the general evolutionary argument. But the merely partial efficiency of these adaptations has not been seen as an active selective force in its own right.

Avian predators, so far as is known, learn to search for their prey within ecological limits doubtless determined by inborn releasing mechanisms, and they must also learn to avoid distasteful species. In an inexperienced predator population, the discovery and consumption of a palatable prey increases the probability that more prey of the same kind will be taken (de Ruiter, 1956: Blest, 1963b), and, if Tinbergen's (1960) hypothesis of "specific search images" retained over relatively long periods by the members of predator populations proves valid (Gibb, 1962), the behavior of the predator populations themselves is altered by experience. It would follow, then, that the existence of specific search images could provide a source of selection pressures promoting divergence within related aposematic forms, if there were even one species of predator for whom the group as a whole proved palatable. For the greater the population density of visually similar forms, the more rapidly will a specific search image directed to them be set up; and this adaptively undesirable consequence of Müllerian resemblance will be attenuated if the similarities are broken down and replaced by divergences in the course of evolution. In fact, the situation would necessarily impose simple direct selection for divergent forms, which might or might not be balanced by the factors promoting Müllerian convergence. The plausibility of this hypothesis can only be assessed when much more is known about the pattern of avian predation in tropical forest. In particular, the relatively narrow ecological niches which are said to be characteristic of tropical birds (MacArthur & MacArthur, 1961; Klopfer & MacArthur, 1961; Klopfer, 1962) may prove to have special selective consequences for insects with spatial distributions which are complex in relation to those of their potential predators.

In contrast to what has been claimed for the saturniids (Blest, 1957), there is no clear derivation for the display behavior of these two families. Although there are rhythmic displays of the type which have been assumed to be transitional between flight behavior and static displays (Blest, 1957), there is no evidence of intermediate types; all the displays are static save for the leg-quivering movements found in some *Bertholdia*, etc., and a few other specialized components, and wing-waving appears only as an accompaniment of walking. The evolutionary origins of the static displays are consequently obscure. Rhythmic displays in which the wings are slowly raised and lowered without locomotion are performed by the North American *Diacrisia virgo*, but nothing can be said about the relationship which they may bear to the present tropical behavior patterns. Nor, as was hoped when the study was started, is the origin and precise function of sound production suggested by comparative study. All that contemporary species seem able to demonstrate is that when unpalatability has reached a certain degree, the capacity to produce ultrasonic sounds is lost, presumably because it confers no further advantage. At present, too little is known about the treatment of unpalatable prey by bats for their significance to be assessed.

9. SUMMARY

1. The protective display behavior of 183 New World species of arctiid and ctenuchid moth from Trinidad and Panama is summarized, and compared with that of the Saturniidae. Their displays are characterized by the independent evolution of a large variety of "specialized" devices, sometimes developed uniquely, but often showing intergeneric convergence. All but a few displays are static and there are virtually no rhythmic components. The most advanced forms seem to rely upon "reflex immobilization" as a defence.
2. Most members of the two groups possess tymbal organs on the metathorax, but only a minority of species use them to produce ultrasonic signals when handled, and the ability to do so is lost in the advanced ctenuchids, although some ctenuchines transitional between the two families (*e.g., Eucereon, Episcepsis*) readily emit sounds. There is no correlation between the mode of protective coloration and the tactile threshold for sound-production, save that in the more palatable phaegopterine arctiids (*e.g., Melese, Bertholdia* and *Ochrodota* spp.) the threshold for sound-production is lower than in those which are more distasteful (*e.g., Automolis, Robinsonia* and *Cissura* spp.).
3. The possible role of environmental diurnal temperature in determining the course of the evolution of protective displays is discussed.
4. It is suggested that the families show less simple Müllerian convergence within genera than might be predicted from their almost uniform unpalatability. Reasons for this are discussed, with particular reference to L. Tinbergen's hypothesis of the "specific search images" of birds.

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11

Observations on the Habits of the Proboscis Monkey, *Nasalis larvatus* (Wurmb), Made in the Brunei Bay Area, Borneo

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(Plates I-IV; Text-figure 1)

THE PROBOSCIS MONKEY, *Nasalis larvatus* (Wurmb) is a distinctive species of colobine monkey restricted in its distribution to the island of Borneo. Little is known of the biology of this species. It does not thrive well in captivity and thus is seldom found in collections of zoological parks. The San Diego Zoo has exhibited five specimens at various times between 1956 and 1963 (Clyde A. Hill, personal communication). One male survived four years. Eight monkeys were obtained by the Surabaja Zoo, Surabaja, Indonesia, in 1961 and all were still alive in August, 1963, when the birth of a baby occurred (Hilmi Oesman, personal communication). Pournelle (Sarawak Museum Journal, Vol. IX, No. 15-16, New Series) refers to records of a proboscis monkey kept in the Giza Zoo, Cairo, in 1899, another that lived 69 days in the London Zoo in 1902, and a third that survived two and a half years in Calcutta in 1902-4.

Perhaps because of its limited range, the monkey has received little attention from naturalists in the field, and as a result there are few data on its ecology and behavior. Much of the information available on the biology of the proboscis monkey under natural conditions is to be found in the publications of Allen & Coolidge (1940), Banks (1931), Chasen & Kloss (1931), Davis (1962) and Hose (1893). Schultz (1942) has described the growth and development of the species.

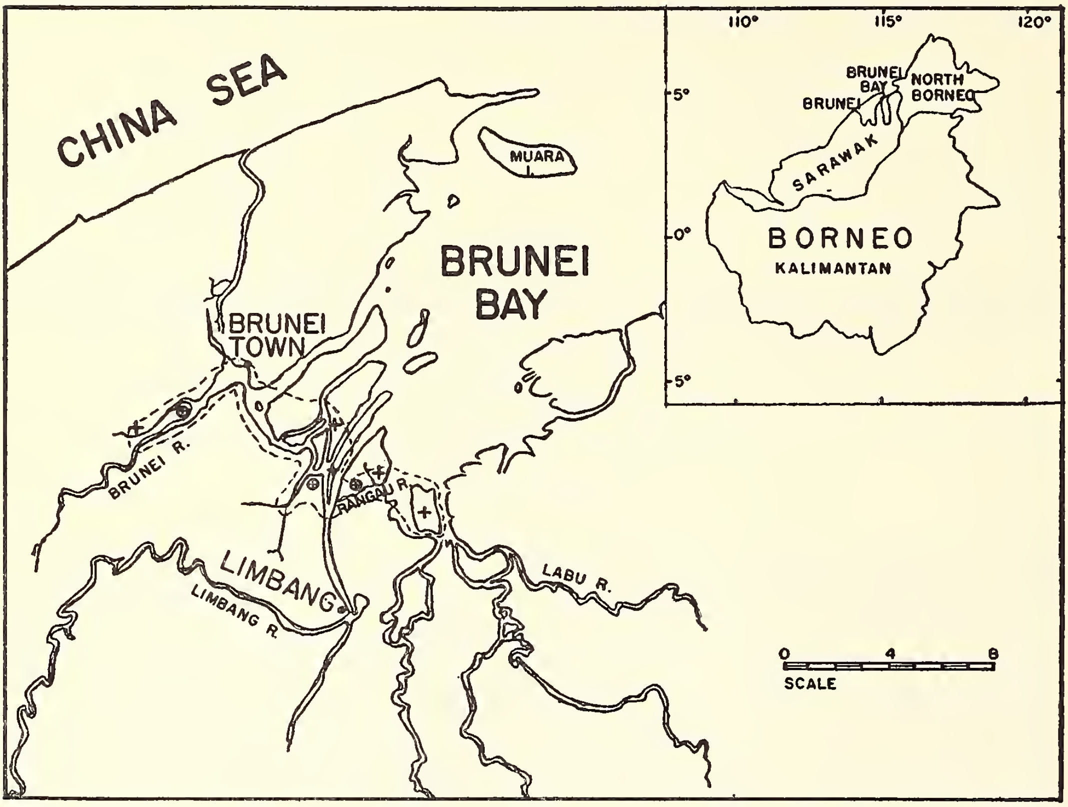
In November, 1962, my wife, Lynn, and I arrived in Borneo to film and study several aspects of the island's natural history. From the middle of November to March 1, 1963, we spent a total of 62 days observing the proboscis monkey. The study was interrupted between December 9 and February 1 by a revolution in Brunei. Text-fig. 1 shows the study area.

Methods

Almost all observations were made in the tidal mangrove swamps around Brunei Bay where the monkeys were common. We averaged six hours a day in proboscis habitats, although on some occasions we spent ten or eleven hours in the field. In the early part of the study, observations were largely carried out between 8 a.m. and 5 p.m., while during February we were normally in the field between noon and 7:30 p.m. The actual time we could see or hear one or more individuals averaged about an hour a day.

Each day the monkeys had to be located. Sometimes this could be done at once; sometimes it took hours, even when we were concentrating on a single troop and knew in which trees they had slept the previous night. But once located, a troop often moved back into the mangroves away from the edge of the stream where it was impossible to follow.

All of my observations were made from an outboard motor boat. Following them on land would have been impossible, since the habitat was an impassable mass of mangrove roots and soft, slippery mud. The noise of a person attempting to penetrate this vegetation would certainly have panicked the animals. By remaining in our boat and thus appearing not unlike the rest of the river traffic—native Malays in outboards (of which there was an overabundance)—we could occasionally approach within thirty feet of the monkeys. If we stayed at a distance of one hundred yards and watched with binoculars, they would tolerate our presence indefinitely. Under such conditions, the monkeys, even if aware of being watched, did not display outward signs of nervousness. Often they would meet the observer's gaze for seconds on end with a generally calm and placid demeanor.



Text-fig. 1. Geographic distribution of the proboscis monkey around Brunei Bay. Dotted line indicates geographic limits of study. Crosses indicate approximate centers of home ranges of the eight troops recorded. Crosses inside a circle indicate the location of the three troops that were observed closely.

Description

The coloration of the proboscis monkey is somewhat variable. The shoulders and upper back are yellowish brown to reddish brown, almost maroon in some individuals. Only the tips of the hairs are so colored, the proximal two-thirds being gray, and this gives a grizzled appearance to the pelage that increases as the pelage wears. The back is yellowish-grayish, forming a lumbar patch sharply set off from the color of the rest of the back and thighs. The tail is about the same color as the lower back, and sometimes, but not always, darker above than below. The belly is yellowish or grayish and sometimes washed with rufous. The legs and feet are yellowish-white and usually distinctly grizzled. A reddish-brown cap on top of the head is continued as a narrow nuchal stripe onto the shoulders. The throat and sides of the neck are cream color, sometimes washed with rufous. The naked parts of the face, including the nose, are approximately hazel in recently tanned skins. Color differences have been used as a primary basis for the recognition of the two subspecies, *N.l. larvatus* and *N.l. orientalis.* Davis (1962), however, has questioned the validity of these forms.

The second and third toes of the hind foot are often webbed to the middle of digit II (Schultz, 1942).

Sexual dimorphism in structure and size is pronounced. The most striking morphological difference between the sexes is the enormously enlarged, bulbous nose of the adult males (Plate I). In females and juveniles of both sexes the nose is snubbed upward but is of normal size. According to Davis (1962), the proboscis of the adult male cannot be inflated but moves considerably with movements of the mouth.

Allen & Coolidge (1940) record a male that weighed 52 pounds, and Schultz (1942) gives the mean weight of ten adult males as 45 pounds and that of females as 22 pounds. Thus, as Davis (1962) points out, males are twice as heavy as females, a sex difference that is equalled among cercopithecids only in some baboons.

In the wild the monkey's large size, long tail and rufous coloration make it impossible to confuse with any other Bornean primate.

HABITAT

The major habitat-type of the proboscis monkey is invariably referred to as the nipa-mangrove association (Davis, 1962). My observations indicate that this habitat designation requires some qualification.

The nipa palm *(Nipa fruticans)* and the most common mangrove *(Rhizophora apiculata)* around Brunei Bay are both plentiful in the tidal regions of Borneo. Plate II, fig. 1, an aerial photograph of a tidal stream, shows some nipa and mangrove in association, but they usually vie for space in continuous patches. One or the other predominates. Also, I found that the monkeys had no use for the nipa, and I seldom found them among its fronds. One might leap into a nipa on its way out of another tree or flee from the water's edge through nipa at the observer's approach, but they showed no tendency to rest or sleep in the nipa or eat any part of this common palm. A list of the more common trees found in the tidal areas around Brunei Bay is given in Table I.

During the day the proboscis monkey's favorite tree is unquestionably the mangrove, while at night it prefers the pedada *(Sonneratia alba).* The dense growth of the mangrove provides protection from the equatorial sun; the upper branches of the pedada afford protection from predators at night.

HABITAT RELATIONSHIPS

We frequently encountered two other common primate species found in association with *Nasalis* in the Brunei Bay area: the silvered leaf-monkey *(Presbytis cristatus)* and the crab-eating macaque *(Macaca irus).*

We found the silvered leaf-monkeys to be shy, somewhat less noisy and much more skittish than proboscis monkeys. They appeared to occupy a similar ecological niche in the Brunei Bay area, but they were not as common here and their troops were composed of fewer individuals.

Troops of macaques were about as plentiful as the proboscis troops, but normally they contained fewer individuals. Therefore, I believe the proboscis monkey was the most common primate in the areas we investigated.

Although the macaques often spent the night in pedada trees as did the proboscis monkeys and silvered leaf-monkeys, they spent their days on or near the ground, apparently feeding on a variety of nuts, fruits and crustacea, thereby utilizing the same habitat as *Nasalis,* but in such a way that competition was virtually non-existent.

Unquestionably the most common mammal in proboscis habitats was the large fruit bat *(Pteropus vampyrus)* that roosted by the tens of thousands on an island—Puala Siarau—in Brunei Bay. We could see them each evening as the sun set fanning out across Brunei in all directions for a night's feeding.

Porpoises were observed in a narrow, muddy tidal stream on one occasion.

Except for the common sandpiper *(Actitis hypoleucos),* waders and sea birds were conspicuously scarce. Several species of land birds, however, were common in the tidal areas. These included the stork-billed kingfisher *(Pelargopsis capensis),* forest kingfisher *(Ceyx erithacus),* broadbill *(Cymbirhynchus macrorhynchus),* imperial pigeon *(Ducula aenea),* broad-billed roller *(Eurystomus orientalis),* green pigeon *(Treron fulvicallis),* Macklot's sunbird *(Nectarinia chalcostetha)* and the sea eagle *(Haliaeetus leucogaster).*

The mangrove snake *(Boiga dendrophila)* was common, and the monitor *(Varanus salvator)* was also observed in proboscis habitats.

TABLE I. COMMON AND SCIENTIFIC NAMES OF SOME OF THE TREES CHARACTERISTIC OF THE TIDAL AREAS INHABITED BY PROBOSCIS MONKEYS IN THE BRUNEI BAY REGION (SOURCE: BERTRAM E. SMYTHIES)

Common Name	Family	Species
Dungun	Sterculiaceae	*Heritiera globosa*
Dungun	Sterculiaceae	*Heritiera littoralis*
Bakau	Rhizophoraceae	*Rhizophora mucronata*
Bakau	Rhizophoraceae	*Rhizophora apiculata*
Bakau	Rhizophoraceae	*Bruguiera gymnorhiza*
Bakau	Rhizophoraceae	*Bruguiera sexangula*
Api-api	Verbenaceae	*Avicenia alba*
Pedada	Sonneratiaceae	*Sonneratia alba*
Perepat	Sonneratiaceae	*Sonneratia caseolaris*
Nyireh bunga	Meliaceae	*Xylocarpus granatum*
Nyireh batu	Meliaceae	*Amoora cucullata*
Nipa, apong	Palmaceae	*Nipa fruticans*

The clouded leopard, *Felis nebulosa diardi,* is said to inhabit mangrove areas (Banks, 1931, 1949). G. S. Brown, former Conservator of Forests, North Borneo, killed one that had, together with a second individual, just killed a large male proboscis monkey near Muniang, at the northern end of the Trusan Kinabatangan, on March 19, 1950. We saw no evidence of this cat in the Brunei Bay area.

Population Density

Our search for the proboscis monkey extended about three miles up the Brunei River from Brunei Town and down river toward Brunei Bay, the Limbang River, and the little water-village of Rangau, a distance of ten miles. I have calculated rather roughly that our investigations covered a total of 14 square miles. However, broad rivers, villages within this area and hills probably accounted for one-half the total, or approximately seven square miles. In the remaining seven square miles, we located eight troops of proboscis monkeys totalling approximately 160 individuals, an average of 20 individuals per troop. This gave a population density of about 23 individuals—slightly more than one troop—per square mile of available *Nasalis* habitats in our study area. There were many sections of the mangrove swamps, however, where we never saw monkeys, so the density in terms of the area actually utilized would be higher than the figures given. In estimating density by this method I would be inclined to put the figure nearer one troop per three-quarters of a square mile of occupied habitat. However, three troops were studied carefully enough over a three-month period so that the boundaries of their home range were known. By estimating distances in our boat, I calculated that all three troops lived within approximately one-half a square mile. This second method of determining home range size seems inherently more accurate than the first, so I have concluded that the average home range is closer to one-half a square mile than three-quarters of a square mile.

By a proboscis troop's home range, I mean an area it inhabits exclusively under normal conditions. We saw no evidence that these monkeys establish a territory and defend it.

Troops of proboscis monkeys have been reported in literature to average about 15 or 20 individuals (Davis, 1962). Among the eight troops we studied, the number of individuals ranged from 12 to 27. Counting had to be done around sundown as the monkeys ascended the pedada trees for the night. Since they often did not begin appearing in the lower branches of the pedadas until one-half hour before sunset and climbed sporadically until almost dark, it was always difficult to get an absolute count. Counting monkeys in mangrove areas without pedadas was virtually impossible. The foliage was too dense and the monkeys were not silhouetted against the sky as they were in the pedada trees. An average-sized troop would generally occupy six or eight 30-foot pedada trees for the night, or two or three larger ones 60 feet or more in height.

On two occasions, however, we observed concentrations of proboscis monkeys in excess of 40 to 50 individuals. One such case occurred on the Labu River in Brunei's smaller enclave. Here, at dusk one evening, we counted 44 or 45 animals and the number actually present could easily have been as high as 50. Local Malays were under the impression that this group could be found in the same trees every night, but it was not observed on a second trip to the Labu region.

In another instance, one of the first days out in our boat we located a troop of monkeys about three miles west of Brunei Town on the Brunei River. At that time we counted 27 individuals. After defining the limits of their home range, we never found them outside these limits. Yet in the dusk one evening in February, a few days before we were due to leave Brunei, we counted about 60 monkeys together in this area silhouetted against the sky. On later occasions we located the monkeys again but could never attain a count of 27. The interpretation of this observation may be that a troop whose presence we did not know of had joined the troop we had previously observed. This was apparently a temporary situation, perhaps for just one night; and when the troops separated, some individuals may have exchanged positions.

Troops of proboscis monkeys appear to be rather loosely organized, at least when compared to the silvered-leaf monkey and macaque species occurring in the same area. Members of the latter species were usually within 50 yards of one another. At night they often slept in one tree, at least in nearby trees. In contrast, I saw a troop of proboscis monkeys spread out for about a third of a mile along a river bank, and in one case observed them in trees for the night about a thousand feet apart.

Proboscis monkeys invariably sleep singly except for mothers with young. With no more than a silhouette to go by, this characteristic behavior distinguishes proboscis monkeys from the other species, although they are also easily identified by their large size and long tails.

Solitary proboscis monkeys appeared to be rare. Only three possible cases were observed during the course of this study. As is the rule in other primates, all of these individuals were males. On one occasion the troop we were most familiar with crossed a stream and began living in a section of the mangrove swamp we had not seen them use before. (Of the three troops we studied carefully, this was the only time we found a monkey outside the limits of the range we had defined for each troop). A few days later we found a full-grown male alone on the side of the stream the troop had left.

In a second instance, we found an isolated male perched in a mangrove overhanging the water. A search through the nearby trees, as we went by in our boat, revealed no other monkeys in sight. We found this male along the Segaliud River which empties into Sandakan Bay. He had a greatly inflamed and obviously infected area around an eye and about half of the cheek. This was the only monkey seen that did not appear to be in good health.

Among one of the other troops we regularly observed, we often found two or three old males apart from the troop high in favorite pedada trees in the heat of midday when the rest of the troop was nowhere in sight, having retired to the shade of the mangroves. These individuals were not solitary but merely peripheral. They regularly spent the night in the same trees as the rest of the troop.

Several incidents that came to our attention indicate that solitary, old male proboscis monkeys may sometimes behave in a very atypical manner. We were told of a huge male that wandered into Brunei Town and was caught by Malays. He was tied up so tightly that he injured himself trying to escape and had to be killed. After we left Brunei we received word that another exceptionally large male had entered Brunei Town. This one was also captured and died about a week later.

A photograph appearing in LIFE (September 14, 1959) shows a huge male proboscis monkey sitting on the edge of a small boat that had been lowered for him from a yacht. According to the caption, the monkey had been found "floundering" in the South China Sea, and after a short rest on the gunwale of the boat, he jumped back into the water. It is possible that this animal could have been swept out to sea accidentally, for around Kuching the tides are swift. The proboscis is, however, a strong swimmer, and I doubt that an adult male could involuntarily be carried far by currents from emptying rivers. Instead, these three incidents may have resulted from varying degrees of dotage in old males.

Old males appeared to dominate to a limited extent the other monkeys in the troop. But the only way I noticed this dominance expressed was by the utterance of a prolonged, nasal snort. Squabbles over trivial things like a position on a branch or a handful of leaves frequently erupted in the group and were invariably accompanied by loud shrieks. At such times one or more old males emitted this lengthy snort, whereupon the group quieted down and the disturbance ceased.

The shrieks of the proboscis and the silvered leaf-monkey are very similar, and we found we were unable to identify the species when we heard only these sounds. However, if the group were proboscis, the nasal snort of an old male would soon be heard.

Except when the old male made his prolonged snort, all animals appeared to behave much alike toward each other. The only specific relationship I observed was between mother and young. I had little chance to study this relationship but noticed that the infant was always carried on the chest, clinging to the mother with all fours if necessary. When a mother sat quietly, the young often moved away from her, but at the slightest provocation it would race back to the female (Plate II, fig. 2).

We were told in Brunei, where shooting monkeys is not prohibited, that if a proboscis monkey were only wounded, others would come to its rescue and carry it off before the hunter could reach it. Although this is possibly true, my own observations provide no verification. In fact, we had one experience that appears to support the contention that the social bonds between members of a proboscis troop are not as strong as in the case of some primates. On this occasion we had panicked an entire troop by running our boat through some mangroves to the base of a huge dungun tree the troop was in. In the confusion and panic, a mother fled, leaving her young stranded on a limb some 30 feet over our heads. Left alone, the infant screamed wildly to be rescued. However, neither the mother nor other members of the troop returned to rescue or defend the infant while we were present.

VOCALIZATION

I have referred to the typical vocalization of the older males as a prolonged, nasal snort. The sound really defies words. There is a hollow resonance to it, and I have been tempted, also, to call it a nasal "growl." It is a lazy sound and lacks urgency or fear, although the old males constantly emit it to register displeasure.

Another characteristic vocalization of the proboscis monkey is a nasal "honk." This sound is given by an adult in time of danger. If the intruder is an unfamiliar sight to the animal, but still at some distance, the monkey might be seen perched with all fours together on a limb, head and shoulders pushed forward and mouth tautly open in a big "O." At such times it may give a few sharp honks, its nose rigidly straightening out and up with each honk.

If the observer can remain hidden until the monkey has approached to within five or ten feet, it may turn and flee without a sound when it discovers the human being. The actions of the monkey will then alarm the others and the whole troop will disappear. If a person in a boat moves quickly under or among a troop, startling them all, there will be a great crashing and leaping of monkeys and swaying and shaking of trees to the accompaniment of loud shrieks and honks. These shrieks are the most regularly heard sound. Whenever the troop is highly excited or frightened, or individuals are fighting, these shrieks will invariably be emitted.

One other vocalization recorded was a contented mewing note I heard given by a juvenile in the Surabaja Zoo while it was eating.

MOVEMENTS

The boundaries of a home range are normally determined by hills or mountains and large streams and rivers. Where tidal lowlands are broad enough, home ranges meet, the boundaries being determined by small streams or a hiatus in the preferred vegetation.

Troops appear to move through their home ranges at random. I have watched a troop spend five consecutive nights in pedada trees within 600 feet of each other, but on the following morning travel more than a quarter of a mile in one hour through the mangroves for no reason apparent to me. This rate was as fast as any I recorded for a similar distance. When frightened, the monkeys were observed rushing away through the trees at almost 150 yards a minute for distances under 100 yards.

Since streams of all sizes lace the tidal swamps to drain the area at low tide, these are frequently crossed by swimming if branches do not reach far enough to permit the monkeys to cross in the trees. Certain points where a tree may hang over the water or where a stream may narrow are preferred crossing places. I discovered four such places among three troops. But except for locating and determining the use of these spots, I could determine no other regularity to their movement, no favorite routes or pathways, no schedule or cycle by which they moved about.

Ivan Sanderson states in "The Monkey Kingdom" that the proboscis monkey "will drive other animals and especially monkeys from territory they consider to be theirs." My observations do not confirm this. I never observed these monkeys defending a certain area, either physically or by vocalization. On one occasion I watched a troop of silvered leaf-monkeys move through the home range of a proboscis troop without incident, sometimes passing within a few yards of proboscis individuals. In "The Monkey Kingdom" and in "Living Mammals of the World," Sanderson also states that proboscis monkeys like to "bask in the sun," indulge in "strenuous exercise" and "regularly go swimming for pleasure." None of my observations support these statements.

ACTIVITY

The monkeys apparently leave their sleeping tree very early. Although once I reached some trees at 6 a.m. where I had left a troop the night before, the monkeys had already left. Three or four other times I arrived about dawn at trees I had seen the monkeys enter the evening before, but never arrived early enough to see them descend the pedadas.

The monkeys eat and are active at all times of the day, but their greatest period of activity is from late afternoon until dark. This is also probably the time at which the greatest volume of food is consumed. During most of the day the monkeys are well hidden among the mangroves and observing them is difficult. With one troop, though, a small navigable stream penetrated its territory, and we could get close to it and film the monkeys during the middle of the day. Often we would cut the motor and tie up to a handy limb and wait quietly, suspecting the monkeys were near. An hour might go by without a sound, and then with luck we might finally hear a shriek nearby, followed by the prolonged nasal snort of an old male.

In mixed cover I never succeeded in determining the kinds of trees in which the monkeys preferred to spend the night. But where pedada trees exist, the monkeys invariably spend the night among their branches (Plate IV). There seems to be good reason for this. The monkeys like the leaves, and food is no more than an arm's length away. Also, since pedadas grow taller than the mangroves, they serve as a convenient look-out. They are generally tallest along the water's edge and here the monkeys most frequently spend the night. No doubt such sites offer the best protection from the clouded leopard.

In times of danger the monkeys are often so situated that they have the option of leaping into the surrounding trees or into the water. Hilmi Oesman, board member of the Surabaja Zoo, has assisted in capturing proboscis monkeys in Kalimantan and explained how it is done. The monkeys are approached at night from the land so that they jump into the water. A semi-circle of men in boats prevents them from reaching the safety of the far shore, and they are captured in the water.

Apparently the monkeys do not normally move about after they have settled down for the night. On one occasion, I made a mental note of the location of several members of a troop at sundown and then returned about midnight with a good spotlight. I could determine no movement about the trees until a few individuals carefully climbed into higher branches as I approached.

LOCOMOTION

In the trees the crashing leaps of the proboscis are similar to those of langurs. We never saw them brachiate, although they might hang idly with one or two hands or move leisurely a yard or so without the use of their feet. When something disturbs a troop and they set off through the trees, the noise of breaking and cracking branches can be heard a long way off. We were often able to locate and identify the monkeys by no more than the sound of breaking branches.

The proboscis appears to have an utter disregard for safety. I have seen individuals drop from high limbs 25 feet to branches below and am confident they did not know exactly where they would land when they left the limb. They will rush onto a dead branch as readily as a live one. When it snaps and falls, they fall with it, looking for the nearest thing to grab in their downward plunge. Accidents, however, appear to be common; healed bone fractures were present in 28% of the 25 skeletons examined by Schultz (1942).

Among the primates, the proboscis monkey is no doubt in a class by itself as a swimmer. It swims with a strong doggy-paddle stroke aided by partial webbing on the hind feet. It also swims well under water. On one occasion, a young adult swimming across a stream dived as we approached in a boat and swam at least 15 feet under water to the bank of the stream (Plate III, fig. 2). On another occasion we witnessed a spectacular diving exhibition by members of a troop crossing a stream. The majority of the monkeys leaped out of the upper branches of a tall dungun tree that leaned over the water (Plate III, fig. 1). By focusing a telephoto lens on one of the branches, I determined its height above the water to be 53 feet. Not all leaped from such heights. A couple of smaller individuals jumped in from the lower branches of a nipa. They leaped out of the tree in such rapid succession that two, and at least once three, were in the air at one time. In less than 30 seconds, the entire troop of 18 or 20 monkeys had plunged from the tree. They fell in an ungainly, spread-eagle position and belly-flopped into the water with a resounding smack. The momentum of the leaps carried them about halfway across the 75-foot-wide stream and they completed the crossing by swimming. I have found no evidence that an act like this has ever been observed before, and was fortunate to have been able to film it with a motor-driven camera. In the 62-day study, we observed a scene of this kind only once. This act, and the fact that we observed proboscis monkeys in the water so seldom, support the view that they swim only when necessary and not for pleasure.

FEEDING BEHAVIOR

While in Kuching. Tom Harrisson told me that the favorite food of the proboscis monkey was the hypocotyl or long bean of the mangrove. We never observed wild individuals feeding on mangrove beans, nor could I induce captive proboscis monkeys at the Surabaja Zoo to accept them, although they devoured the mangrove leaves. Sanderson states in "Living Mammals of the World" that proboscis monkeys feed upon "certain leaves notably bamboo." Bamboo is uncommon in the lowland areas around Brunei Bay, and the monkeys were never observed in or near it.

My observations indicate that at least 95% of the diet of the proboscis monkey consists of leaves. The pedada leaves are probably favored, but mangrove leaves are eaten regularly. In fact, because of the abundance of these trees, mangrove leaves probably make up a larger share of the monkeys' diet than any other variety.

I believe the monkeys do much greater damage to the pedadas than to the mangroves. The pedadas are generally tall and rise above the mangroves and nipas and are a favorite place to sleep. Though the monkeys eventually circulate through their entire home range, they may spend as much as a third of their nights in a particular group of trees. Here, for two or more hours every evening, the troop may feed on the leaves. In three months' time we saw one group of trees become noticeably thinned out. Some of the branches were completely stripped. Intensive feeding on the pedadas may result in the death of the trees. We saw pedada trees in various stages of decimation, from the fully-leaved trees to dead ones, within a few hundred yards of each other in areas frequented by the monkeys.

The monkeys will eat the pedadas in all stages of growth, but this is not the case with the mangrove. *Rhizophora apiculata* is the most common species around Brunei Bay. The new leaf sheath of this species forms a long, thin, bright red cone which the monkeys ignore in feeding, preferring the thicker leaves around it. Thus, this mangrove is not stripped of its leaf-buds.

Within the thick groves of mangroves, there is little variety in the choice of leaves, but along the stream banks there is a wider selection. Here, where they often feed near the ground, we observed them eating tender vines and shoots as well as leaves, but still rejecting many of the plants at hand.

In addition to leaves, the proboscis monkey also probably consumes some fruits and flowers. The fruit of the pedada is a likely candidate, for I observed a captive do so, but I never observed wild monkeys eating it. They were never observed feeding on flowers, either, but the tidal swamps around Brunei Bay were almost devoid of flowers from November through March.

It has also been suspected that the proboscis monkey will eat the huge, round, segmented fruit of the nipa palm, but I am inclined to doubt this. The fruit may be too coarse and too difficult for the monkey to get at. I did discover a large orang-utan pulling one apart on the Segaliud River.

In feeding, the monkeys often take a leaf into the mouth directly and bite it off. The movements are executed in a leisurely manner. If the food is several inches from their mouth, they may choose to pluck off a few leaves at a time or pull the branch over and chew off the leaves, stuffing them into their mouth with their hands if the volume requires it. The feet may also be used for pulling in branches, although I never saw them using their feet to put leaves into their mouth. In the typical feeding position, the monkeys face away from the trunk and toward the foliage on a horizontal limb. Holding onto a branch with one hand, they reach out in all directions with the other.

Status of the Species in Borneo

Several factors contribute to the protection of the proboscis monkey in Borneo. The species is legally protected in Sarawak, North Borneo and Kalimantan. In Brunei, although the Chinese consider the meat a delicacy, few will stray far from their shops to hunt the animals. The Malays are not hunters, either. In fact, we never saw anyone hunting the monkeys in Brunei, although it is not illegal.

The occurrence of the proboscis monkey in a relatively inaccessible habitat that has little economic value to man is perhaps the best guarantee of its survival.

Summary

Sixty-two days were spent studying and filming the proboscis monkey in Borneo. Observations were made from a small boat along tidal streams around Brunei Bay. For about one hour a day we were within sight or sound of one or more monkeys.

Proboscis monkeys live in troops and occupy home ranges of one-half a square mile or more. The troops are loosely organized and interchanges of individuals between troops was indicated.

Proboscis monkeys are large, heavy-bodied monkeys. Older males can weigh over fifty pounds, more than twice the weight of the average female. They move about with typical langur-like leaps, especially when frightened. The monkeys seldom seek the water, but are excellent swimmers both on the surface and under water. They are inveterate leaf-eaters. Probably less than 5% of their diet is composed of other vegetation.

Proboscis monkeys are the most plentiful monkeys in the Brunei Bay area. Their preference for mangrove swamps has not placed them in conflict with man and is their best guarantee of survival.

Acknowledgments

Much of the success of our proboscis monkey project can be credited to my wife, Lynn. She withstood blistering heat, mid-day downpours, hours on a cramped boat, mosquito and fly bites (91 on one leg at one count) to continually assist with the photography, note-taking and maneuvering of the boat.

To Tom Harrisson, Curator of the Sarawak Museum, I owe the suggestion of Brunei Bay as the location for our work. Bertram Smythies, Conservator of Forests, Sarawak, kindly verified my information on trees around Brunei Bay and supplied the data in Table I. Clyde A. Hill, Assistant Curator of Mammals at the San Diego Zoo, supplied information on the history of captive specimens at the zoo. Hilmi Oesman, board member, Kebun Binatang Surabaja, (Surabaja Zoo), Surabaja, Indonesia, graciously acted as host while I visited the Surabaja Zoo.

But my observations would still be in an unpublished form if I had not received the generous assistance of Dr. James Layne, Cornell University. Dr. Layne suggested the skeletal arrangement for this paper and was an enormous help in recommending the omission of data he thought extraneous and the elaboration of data, where

possible, he thought important. Responsibility for the data, however, remains mine.

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EXPLANATION OF THE PLATES

PLATE I

FIG. 1. The only adult male proboscis monkey in captivity in 1963. Photographed at the Surabaja Zoo, Surabaja, Indonesia.

PLATE II

FIG. 1. Nipa palms and mangroves grow along one of the tidal streams that empties into Brunei Bay.

FIG. 2. Adult female watches photographer as her young dangles from a nearby branch.

PLATE III

FIG. 1. Proboscis monkeys leap toward the opposite bank of a stream from limbs up to 53 feet above the water.

FIG. 2. A young adult swims a tidal stream.

PLATE IV

FIG. 1. Proboscis monkeys in the tops of pedada trees at sundown. Three of eight monkeys are clearly visible.

OBSERVATIONS ON THE HABITS OF THE PROBOSCIS MONKEY, NASALIS LARVATUS (WURMB), MADE IN THE BRUNEI BAY AREA, BORNEO

FIG. 1

FIG. 2

OBSERVATIONS ON THE HABITS OF THE PROBOSCIS MONKEY, NASALIS LARVATUS (WURMB), MADE IN THE BRUNEI BAY AREA, BORNEO

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12

Gonadal Hydration of Carp *(Cyprinus carpio)* and Goldfish *(Carassius auratus)* After Injections of Pituitary Extracts[1]

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(Text-figures 1-15)

INTRODUCTION

THE pituitary of carp contains a principle controlling the preparation of semen for spawning in carp, trout *(Salmo gairdneri)* and a number of other species of fish. Clemens & Grant (1964) demonstrated that changes in the water and salt content and sperm cell densities of semen occur in direct proportion to dosages of water extract from whole ground acetone-dried pituitaries of carp and that these responses, being relatively rapid and sensitive, provide an assay for following the activity in chemical isolation and purification procedures. Although the seminal fluidity response provides advantages, such as an hourly pattern of response changes in the same test animal, it is impractical to use with small fish where the semen cannot be collected in sufficient quantity, and gives no biological comparison in female fish.

Since our main purpose was to pursue the role of gonadotropins in the reproductive cycle of fish, we needed a method of measuring a response to pituitary extracts giving a reliable comparison among fish of different size, maturity and sex. We needed to know further the role of electrolytes and water in the reproductive cycle in nature. The phase reported here reveals that water and electrolyte changes can be induced in the gonads of either sex and that these changes are an essential part of the reproductive cycle, in particular, the spawning and prespawning gonadal processes. Data currently collected, to be presented in an additional paper establishing the existence of a water and electrolyte cycle in fish gonads in nature, along with the experimental data presented in this paper, offer convincing evidence that the gonadal hydration of fish is hormonally controlled and regulated by the pituitary gland.

METHODS

Both species of experimental fish, carp and goldfish, were maintained on a year-around basis in the University of Oklahoma Fisheries Research Center ponds which provided us with fish of known history. Fish were seined from the ponds and acclimated in 100-gallon metal troughs at an experimental temperature of 22 ± 0.5° C for a period of one week to ten days before an experiment. Fish were fed (3% body weight) a commercial fish food (37% protein) five days a week. Feeding was discontinued 24 hours before the experiment. Only mature, healthy specimens were used.

Acetone-dried carp pituitaries were purchased for experimentation from Stoller Fisheries, Iowa. Whole pituitaries were ground, extracted with distilled water and injected intraperitoneally. The injection material was prepared as follows: Male and female carp pituitary glands collected in January and dried with four 12-hour changes of acetone were crushed, extracted with distilled water at room temperature for 30 minutes and filtered. The filtrate solution was then diluted with distilled water to the desired concentrations based on the dry weight of the pituitary glands. Dosages were made in logarithmic proportions and administered on a weight basis, *i.e.*, x mg of acetone-dried, whole pituitaries per 100 gm of fish body weight (excess water blotted from its

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[1] This study was supported by Grant A-3445 of the National Institute of Health.

surface). Extract volumes were injected proportional to fish weights, 1.0 cc/100 gm respectively. Gonads were weighed to the nearest gram and "fat-free" pieces (250-650 mg) were placed on tared glass cover slips, macerated and weighed to the nearest 0.2 mg. The weight lost on drying overnight at 65° C was accepted as the water loss.

Deviations from the above practices are mentioned in the text.

RESULTS

Seminal Response

The seminal fluidity of carp (groups of five fish, 450 ± 50 gm) peaked approximately 24 hours after injection (homoplastic pituitary extract), then gradually subsided until the base level was reached some three to four days later. The curve is skewed to the left, indicating that for *ca.* 24 hours after injection there was a more rapid movement of water into the testes (semen) than out.

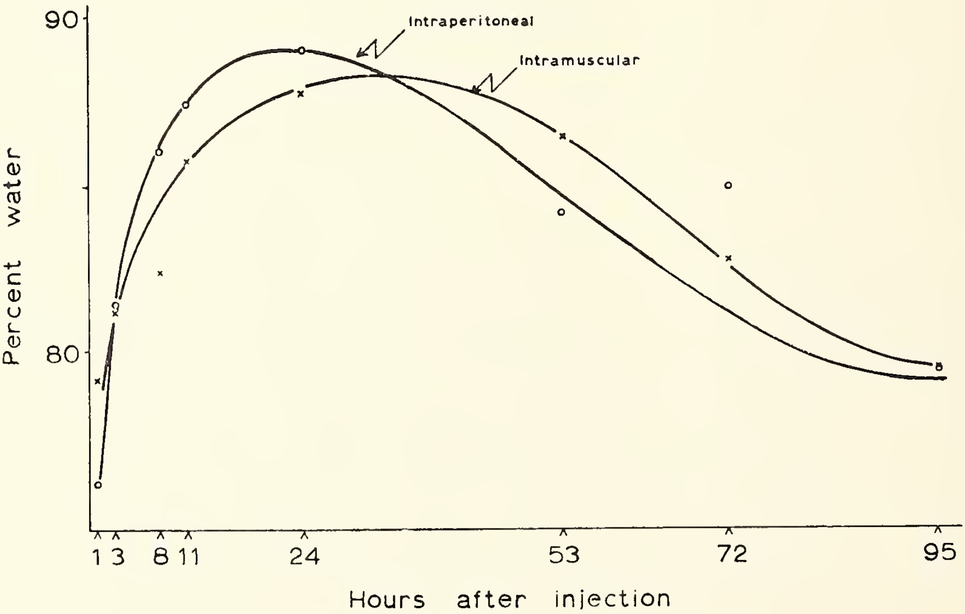
The experiment exemplified by Text-fig. 1 was conducted to determine whether the muscle or the body cavity was the more suitable site for injection. The levels of response in the two types of injections were not significantly different.

Testicular Response

A testis was removed from a mature carp injected 24 hours previously with a water extract from 1.0 mg of whole ground acetone-dried carp pituitaries. Its length was divided into ten parts and the relative water percentage was determined for a fraction of each part. Values for all ten parts of the testes were similar with the exception of sample eight which was apparently a technical error. A gain in water content appeared throughout the entire length of the organ and no part of the testis contained more water than another in gross analysis (Table I). Since the testes appeared uniformly hydrated in our experiments, it remained then for us to establish whether or not testicular hydration could be correlated with hormonal injections and the seminal fluidity response.

The water content of both the semen and the testes of injected fish increased proportionally with the dosage (0.69 to 69 micrograms/gm range with half-log dosage increases) (Text-fig. 2). The relative water content of the testes was lower and results were more uniform between dosages. This demonstrated that the seminal fluidity response was reflected in gonadal hydration and that the testes could be used to measure the hormonally induced response.

Since this experiment, several hundred male goldfish have been used throughout the course of the year in our assay work. The data for these fish were used in determining the standard curve (Text-fig. 3). The assay range is represented by the straight part of the sigmoid curve between 1.0 and 100 μg/gm (0.1 and 10 mg/100 gm in practice). The standard deviation, standard error



TEXT-FIG. 1. The mean (five carp) seminal hydration response with respect to time after a single injection of homoplastic pituitary extract.

Table I. The Water Percentages of Ten Portions of Carp Gonad Equally Spaced Throughout Its Length, with Respect to the Duration of Drying

Part No.	6 hrs.	24 hrs.	72 hrs.	96 hrs.	120 hrs.	144 hrs.	Mean[1]	SD
1	80.4	79.5	79.2	79.9	80.8	80.3	79.9	0.64
2	75.7	78.8	78.8	79.3	79.4	79.3	79.1	0.30
3	75.7	79.3	79.4	79.2	80.0	79.6	79.5	0.32
4	77.8	78.6	78.6	79.9	79.9	79.5	79.3	0.66
5	78.7	79.9	79.9	79.5	80.0	79.9	79.8	0.20
6	78.7	79.6	79.3	80.7	80.3	79.6	79.9	0.58
7	77.5	78.2	77.2	77.3	78.5	78.3	77.9	0.61
8	70.2[2]	71.3	71.0	72.6	73.0	72.6	...	...
9	77.9	79.2	78.9	78.7	79.5	79.6	79.2	0.38
10	74.7	78.6	78.6	78.5	79.5	79.0	78.8	0.42
Mean	77.5	79.1	78.9	79.2	79.8	79.5		
SD	1.80	0.87	0.88	0.97	0.65	0.54		

[1] 6-hour readings omitted.

[2] Apparently an original error in weighing, calculations omitted.

and the number of fish for the controls and each of the dosages were respectively as follows:

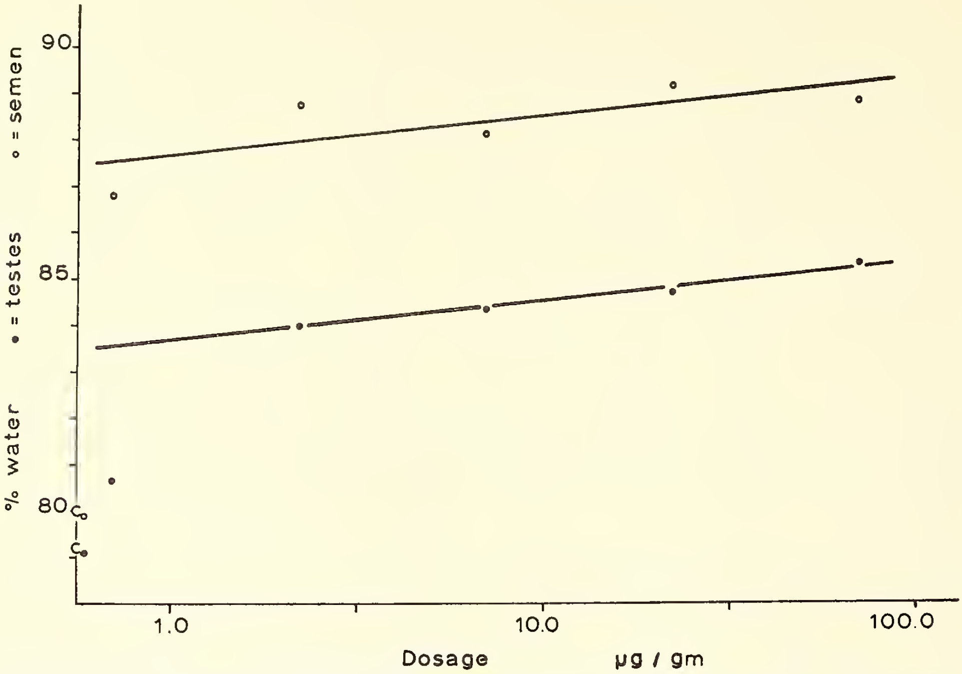
Control	2.46,	.26,	140
0.2 μg/gm	1.79,	.25,	52
0.6 μg/gm	2.09,	.35,	36
2.0 μg/gm	1.76,	.22,	62
6.6 μg/gm	2.24,	.54,	17
10 μg/gm	2.58,	.53,	24
22 μg/gm	2.21,	.27,	68
31.6 μg/gm	1.99,	.48,	17
50 μg/gm	1.47,	.49,	9
100 μg/gm	1.16,	.24,	24
316 μg/gm	.63,	.28,	5

The standard deviations are considerably higher than for each individual experiment since there was a seasonal response variation in the controls and in the experimental fish which approximated the seasonal gonadal water of fish in nature (Text-fig. 4).

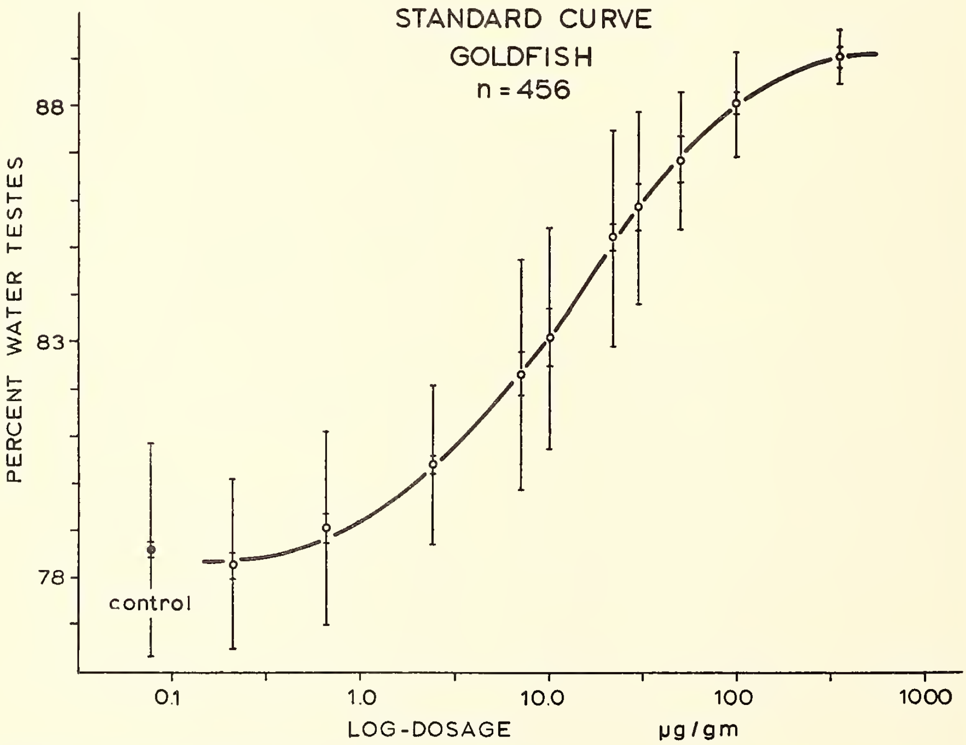
The standard curve represents the entire year. The periods of greatest response appeared during May, June and October. The period in the fall should be considered late September and early October, since the October experiments were conducted during the first ten days of that month and the September ones at the end of the first week of the month. During the spring period of April, May and June, the spawning period, the experimental fish gave the greatest response over that of the controls. For example, the difference between the average monthly response for an injection level of 22 μg/gm and the controls was 6.3 in February, 6.5 in March, 13.0 in April, 11.7 in May, 10.2 in June, 5.3 in July, 5.8 in August, 2.8 in September, 8.6 in October and 8.6 in November. The greatest difference between the maximum and the minimum monthly average during the year was 6.9%; and for the experimental fish receiving 22 μg/gm was 3.3%. The respective standard deviations and errors were 2.5 and .26, and 2.2 and .27. There was greater seasonal variation in our controls than in the injected fish.

The controls showed a significant reduction of gonadal water below the pondfish during the period from April through June, while the two values were approximately the same during the rest of the year. This suggested that some aspect of "handling" or perhaps temperature might be involved. However, the period from October to May when fish were moved from the colder pond water to the warmer experimental water (22° C) included times when the control fish approximated the values of the experimental fish as well as the times when the gonadal water of the control fish was significantly lower than that of the pondfish. The change of temperature from the pond environment to the experimental conditions, therefore, did not seem to be a major factor in explaining the reduction of gonadal water of the controls below the gonadal water of the pondfish.

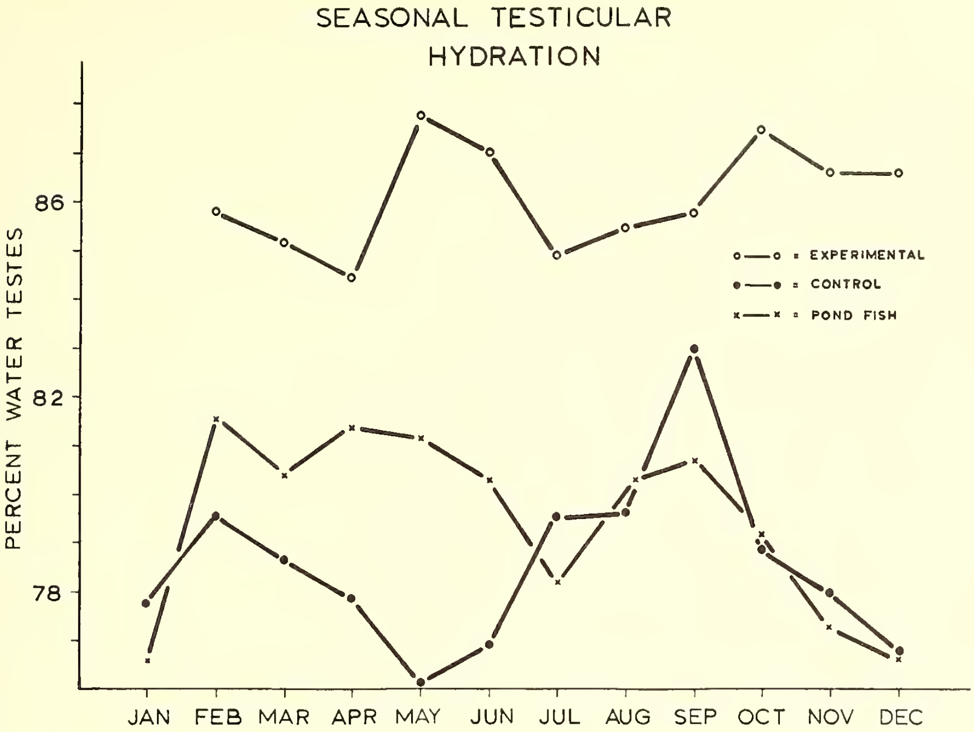
During March, an experiment was conducted to determine the effect of "handling" on the gonadal water of 35 male goldfish. The effects of bringing goldfish from the pond into the laboratory and raising water temperature alone caused a slight decrease which was not statistically significant, while the effects of bringing the fish into the laboratory and raising the water temperature along with the handling involved in their weighing, sexing, fin-clipping and needle puncture caused a 1.6% rise in the gonadal water content ($p = .05$). This was the only step in the handling



TEXT-FIG. 2. A comparison of seminal and testicular hydration, 24 hours after injections of various dosages of homoplastic pituitary extract.



TEXT-FIG. 3. Standard gonadal hydration response curve of male goldfish to injections of carp pituitary extract. Standard deviations and errors are given.



TEXT-FIG. 4. Seasonal gonadal hydration of male goldfish in nature and in injected and non-injected experimental fish.

procedure that produced a significant change in the gonadal water content, but this left unexplained the question of why the controls during the spring months were lower than the pondfish.

Another experiment was conducted on 90 male goldfish at the end of April to determine the effects of acclimation under laboratory conditions. The gonadal water of fish removed from pond water at a temperature of 13° C and acclimated at 22° C under laboratory conditions without food declined from 81.9% to 80.6% in five days, to 78.5% in seven days, to 75.0% in 14 days and to 72.9% in 23 days. When food was given, the gonadal water did not change for a test period of the first five days in the laboratory. Fish kept for seven days without food took approximately 16 days on a *daily* diet of 3% per body weight of Clark's trout food (37% protein) to recover the original gonadal water content. These experiments indicated that food was more critical than temperature in our method of handling.

The effect of food on the gonadal hydration re-opened the question of whether or not our laboratory fish were properly fed. Since feeding procedures were the same throughout the year, the most logical explanation lies in the fact that fish during the spawning period require more food than during other times of the year. In another experiment in our laboratory, goldfish that had shown weekly body gains for seven months on a 3% diet (fed five times a week)[2] at a constant temperature (22 ± .5° C) lost weight during the period from the middle of February to the middle of March. The condition was corrected by raising the food to 5% of the body weight[2]. It is unfortunate to have to report that the control fish which were being fed five times a week on a 3% diet were not changed to a 5% diet at this time.

Male goldfish were collected in the pond on March 1, when the temperature was 3.0° C. Groups of 15 were placed in each of the following temperatures: 3.0, 10.0, 16.0 and 22.0° C (± 0.5) and allowed to acclimate for four days before being injected with water extracts of carp pituitaries in dosages of 316, 100, 10, 1.0 μg/gm and controls. The relative water percentage of the testes was measured 24 hours after injection (Text-fig. 5).

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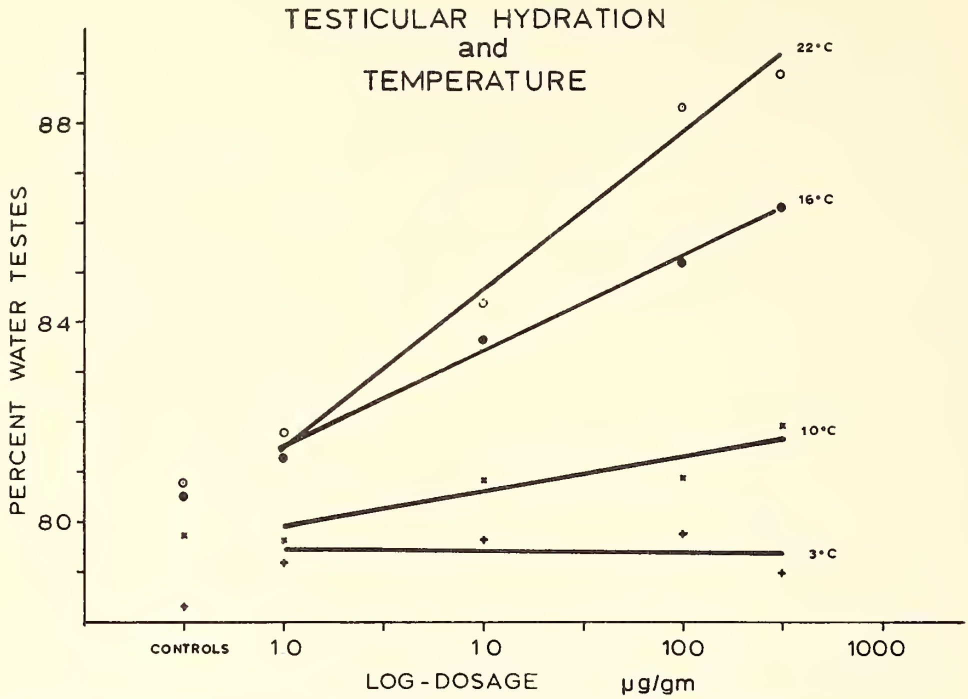
[2] Fish fed five times a week on a 3% diet average 2.1% per day per week and the 5% diet reference is also on a five day basis, which then reduces the diet to 3.6% per day per week.



Text-fig. 5. Gonadal hydration response (24 hours) of male goldfish at different temperatures; injected with carp pituitary extract.

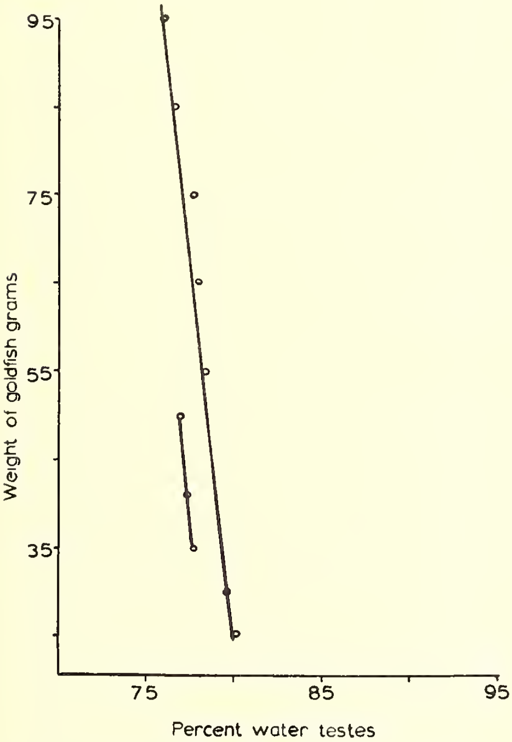
The most apparent observation in Text-fig. 5 is that temperature changes the slope of the curve, which was unlike some of the other varied parameters where the slope remained the same but the level of the curve was uniformly depressed. As was expected, as the temperature increased the response increased and the larger dosages became more effective. Although the control fish had the lowest gonadal water at the coldest temperature, it was not statistically significant. This may mean that the endogenous blood level of the hormone in question was low and that at low blood levels temperature was not a significant factor. However, many other possibilities exist, particularly in the area of inhibition. No response was seen for any dosage at 3.0° C, a weak response ($.20 > p > .10$) at 10° C, while marked responses were observed at 16.0 and 22.0° C. Generally speaking, the response for a six-degree change in temperature between 10 and 16° C was about twice the response for a six-degree change of temperature above and below these values. From a practical standpoint dosages were more critical at the higher temperatures. This observation was in keeping with the fact that goldfish spawn in the spring during the time of the year when the temperature is increasing. Thus, if the stimulus for hormonal secretion were present to provide relatively high blood levels, it would not be effective at low temperatures and, under these circumstances, raising the water temperature would be sufficient to induce spawning.

Some assessment of the effect of various factors on induced and non-induced gonadal hydration, such as environmental variations in illumination and dissolved oxygen, as well as variations in types of injections, gonadal fat and forced exercise (physical stress?), was made from the results of pilot studies and more extensive experiments in certain cases.

Male goldfish exhibited a lower gonadal content when kept in the laboratory under continuous light (71.2 and 73.4%) than in total darkness (75.05 and 75.7%) regardless of whether they were maintained in cold (5-7° C) or warm (22° C) water. The testicular water of those fish kept in the dark was not significantly different from pond fish at the end of the experimental period. Continuous light (200-watt bulb about 30 cm above water 25 cm deep) may have kept the fish active throughout the exposure, thereby increasing their food requirements. We think the decreased testicular water of the light-controlled fish resulted from reasons similar to those found in the fish acclimated without food (p. 197).

Circumstantial evidence indicates that in conditions of insufficient dissolved oxygen the response is lowered. Sixty male goldfish, ranging from 22 to 102 gm, were each injected with the same dosage (0.5 mg per fish) and the testicular water percentages were determined 24 hours later. The data plotted in Text-fig. 6 form two parallel lines. It is postulated that the smaller curve, representing 26 fish, resulted from conditions of low oxygen, since these fish occupied the three compartments towards the discharge end of an overloaded tank through which water was circulated.

Fat deposited around the gonad substantially reduced the water content, and therefore was carefully dissected away from any portion of the gonad used in water content determinations. As a precaution, any fish whose gonadal portion appeared oily after oven-drying was excluded from the determination, since such specimens usually had water contents as much as 3% lower than the mean. On the other hand, we did not use fish that were thin and obviously in poor health, since it was an unacceptable practice to use such individuals even though their gonadal water was only about 1% higher than the mean. However, their muscular water was as high as 7% above the mean.



TEXT-FIG. 6. Gonadal hydration response of varying of male goldfish injected with the same dosage of carp pituitary extract. The small curve is believed to show the effect of low environmental oxygen.

This information should serve to emphasize the importance of using healthy fish of known history from the same population in assay work. In the event that different populations are used, the reference curve should be re-standardized.

The effects of non-hormonal substances injected into the body cavity were to lower the response if an effect was observed. For instance, phosphoric acid buffered slightly akaline lowered the gonadal water content of the controls from 82.7 to 81.0% ($p = .005$). However, in another experiment the experimental fish with 79.5% gonadal water were not statistically different from the controls with 80.0% gonadal water. In another experiment the non-buffered phosphoric acid used to carry P^{32} did not alter the gonadal water (74.3 and 74.2%). Perhaps this means that when the endogenous values are high, it is easier to lower the testicular water content by stress than when the hormonal levels are relatively low.

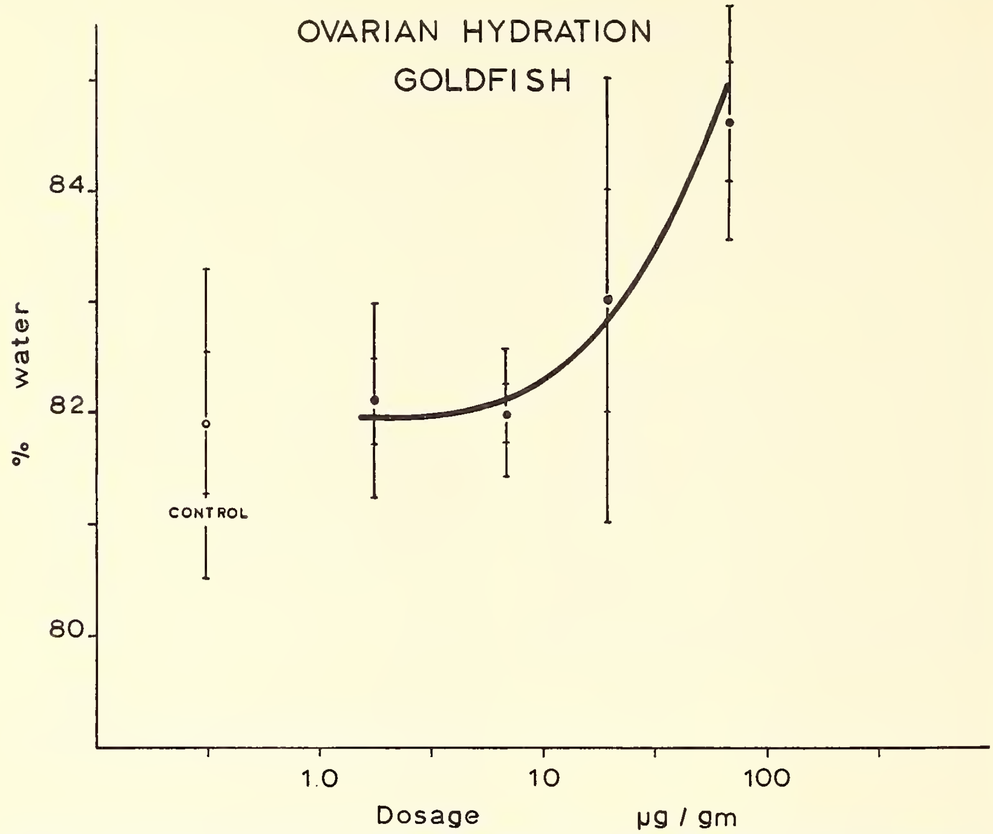
The distilled water used to extract the pituitary activity and to serve as an injection carrier is not in keeping with the osmotically balanced physiological saline normally used for injections. This practice began several years ago (Clemens & Sneed, 1962). No detectable change in gonadal hydration was elicited from a single intraperitoneal injection of distilled water in the amounts we used (up to 1.0 cc/100 gm). Further, distilled water is a more selective method of extraction than water containing electrolytes (Clemens, *et al.*, 1964).

In a series of pilot experiments where carp were forced to swim against various current velocities, the gonadal water content was proportional to the amount of exercise for a given peiod of time, as much as 5% in seven hours. A 5% change in gonadal water is about half the response we are able to induce with injections of hormones.

Ovarian Response

Ovaries of intact goldfish responded with an increase in water when injected with the same pituitary materials as the males (Text-fig. 7). The range of dosages used here tends to suggest that the response was in the "below optimum" range and that higher dosages would be necessary to produce an "optimum response." This experiment was conducted in September when the gonadal water of the female is high. As the spawning season approaches, goldfish ovarian water content decreases.

An increase in ovarian water was found to be



Text-fig. 7. Gonadal hydration of female goldfish 24 hours after injections with carp pituitary extract. Standard deviations and standard errors are given.

associated with ovulation. Specially selected, "ripe" female goldfish injected with pituitary extract in February showed higher water contents than the controls; the ovulated ovaries exhibited higher water contents than those that did not ovulate[3] (Table II). The water content of the ovulated ovaries was 73.0% or above. The average ovarian water content of 35 fish that had ovulated during the course of several experiments was 75.5% (standard deviation, 2.9, standard error, .084), while 17 control fish had an ovarian water content mean of 67.9% (SD 4.365). Eight ovulating females captured in the pond in the act of spawning had an ovarian water content of 81.3% (SD 3.97, SE, .496), while nine non-spawning females taken from the pond one day later had an ovarian water content of 65.2 (SD 1.29). Thus, in both instances, ovulating females had higher ovarian water. It appears that a water increase is a part of ovulation and that this process can be stimulated by the injection of pituitary extract.

Immature Fish Response

Since the water content of the ovary decreases with the degree of ovarian development, immature females possess relatively high ovarian water. The injection of pituitary extract into immature female goldfish produced a variety of responses assumed to be related to the degree of maturity. Gonadal hydration of immature ovaries has decreased with the dosage in some experiments, increased in others, and given little or no response in still others, leaving the hypothesis of the role of hydration of immature ovaries still in a formative stage.

Likewise, the gonadal water content of immature male goldfish does not conform with that of mature specimens. Therefore, immature fish should not be used in the same experiment with adult specimens in bioassay work.

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[3] When female goldfish ovulate, the eggs are released into the confines of the ovary and during the spawning act the eggs are emitted via oviducts. Since the eggs are not released into the body cavity, it was therefore possible to determine water content of the ovaries after ovulation and before spawning.

Table II. The Difference Between the Water Content of Ovulated and Non-ovulated Ovaries in Goldfish

Dosage μg/gm	% H_2O Ovaries	Ovulation	Remarks	G.S.I.
20	69.3	no		.123
20	73.9	yes	complete	.131
20	73.0	yes	partial	.158
10	75.3	yes	complete	.166
10	74.8	yes	complete	.189
10	72.6	no		.163
0	67.0	no		.168
0	67.2	no		.165
0	69.4	no		.176

Effect of Handling on Gonadal Hydration

Mature goldfish averaging 42 gm and mature carp averaging 190 gm were taken on March 6 from one of our ponds with a water temperature of 4.0° C, and allowed to acclimate to 21.0° C over a period of several hours. The fish were left one week in the environmental conditions in which the experiment was to be run.

The experiment was designed to provide controls of four types, each establishing an important reference point along the progression of experimental procedure. The dummy-injected animals were intended as the direct control on the experimental fish. These fish received completely identical "handling," including the shock of needle puncture and the carrier injections of distilled water. Carp were handled in precisely the same manner and at the same time as the goldfish, although the handled controls received no needle puncture.

The handled fish were intended as a control for all aspects of handling on the dummy-injected fish, including the shock of needle puncture but not the distilled water injection.

The "undisturbed" fish were intended as a control for handling on the handled fish. These fish were collected at the same time as the dummy-injected, handled and experimental animals. At this point, however, they were not fin-clipped, weighed or sexed, but were expeditiously placed into a separate tank beside the experimental and other control tanks with temperature, illumination and number of fish identical. On inspection day, fish were drawn with minimum handling and killed until eight males and eight females were obtained. No last minute difference in handling, such as attempts to sex these fish by hand-stripping, was introduced.

The pond fish were intended as a control for the undisturbed fish. These fish were sacrificed after being brought into the laboratory late one afternoon and kept overnight in circulating pond water.

Index cards were marked with the sex, dosage and fin-clip. The cards were mixed and used randomly to assign the treatment for each fish. When killed, the fish were taken randomly from the various tanks a few at a time so that no one individual or group had extra time in the tank. Three levels of injection were selected. The top level, 10 μg of dried pituitary powder per gm body weight, which was known to be a reliable minimum dosage, and the two levels below this (1.0 μg and 0.1 μg/gm) were questionable since they were considered to be above the response threshold of some fish and below that of others. It was thought that these dosages might produce greater variation than higher hormone levels and would be useful in variance analyses.

Carp received 0.01 cc/gm, while the goldfish received half that volume. Both were injected intraperitoneally in the axis of the pelvic fin. Twenty-four hours after injection the gonads of all fish were extirpated and a portion was used to determine loss of weight on drying, which was accepted as the relative water content.

Goldfish Females.—There appeared to be no significant difference in the ovarian water content of females receiving a distilled water injection and females receiving only a needle puncture (Table III). For this reason it appears that the carrier-injected or the handled fish could serve as controls.

An appreciation of the effect of handling is obtained from a comparison of the ovarian water content of the handled fish and the undisturbed fish (Text-fig. 8). Apparently, handling subjects the fish to sufficient stress to cause a significant reduction (2.7%) in ovarian water. The ovaries of females receiving 10μg/gm were hydrated a significant amount over those of any other group. This amounted to 2.6% over the pond females which were the next highest group, and—more significantly—7.2% over the carrier-injected controls.

The effect of the pituitary extract apparently countered the effect of the stress. The 0.1 μg/gm and 1.0 μg/gm dosages seemed to offset the effect of stress from handling, since no significant change in the ovarian water content nor significant variability from fish to fish was found between the fish injected at these levels and the undisturbed controls.

Goldfish Males.—In some respects, similar effects were observed in the male (Table III). There was no significant difference in the testicular water content between the pond group and the undisturbed group, nor between the handled

TABLE III. GONADAL WATER PERCENTAGES OF CARP AND GOLDFISH UNDER DIFFERENT EXPERIMENTAL TREATMENTS

	Male Carp				Male Goldfish			
Treatment	% H_2O	Variance	No. Fish	"t" .05 level	% H_2O	Variance	No. Fish	"t" .05 level
Pond	74.6	2.198	11		79.0	0.737	10	
				+				—
Undisturbed	77.9	0.280	8		78.8	3.691	9	
				—				+
Handled	77.4	1.590	8		80.4	1.091	8	
				—				—
Dummy-injected	77.3	1.262	7		79.9	1.211	8	
				—				—
0.1 μg/gm	77.6	5.368	8		79.0	1.810	9	
				+				—
1.0 μg/gm	80.8	3.828	10		79.4	1.096	10	
				+				+
10 μg/gm	83.9	6.603	8		83.1	3.082	10	

	Female Carp				Female Goldfish			
Treatment	H_2O %	Variance	No. Fish	"t" .05 level	% H_2O	Variance	No. Fish	"t" .05 level
Pond	68.4	3.736	6		72.1	7.727	9	
				+				—
Undisturbed	65.5	1.308	6		70.4	12.623	8	
				—				+
Handled	66.3	1.250	8		67.7	4.404	8	
				+				—
Dummy-injected	64.9	1.905	7		67.5	1.694	8	
				—				+
0.1 μg/gm	66.3	3.096	11		69.8	9.863	10	
				+				—
1.0 μg/gm	68.3	3.683	8		68.9	13.112	10	
				+				+
10 μg/gm	70.7	7.516	7		74.7	13.747	7	

group and the dummy-injected group. Male fish showed a significant increase in gonadal water at the highest injection level, as did the females.

However, the effect of handling appears to cause a response in the male opposite to that in the female (Text-figs. 8 & 9). Gonadal water was significantly more (1.6%) in males and less (2.7%) in females after handling.

Carp Females.—The ovarian water content of the dummy-injected carp was significantly lower (1.4%) than that of carp receiving the same treatment, excluding needle puncture and distilled water (Table III).

A significant decrease (2.9%) in the ovarian water content was found in females brought from the pond into the laboratory and subjected to temperature and minimal handling (Text-fig. 10).

However, we detected no measurable differences in the ovarian water content of minimally handled fish and those experimentally handled.

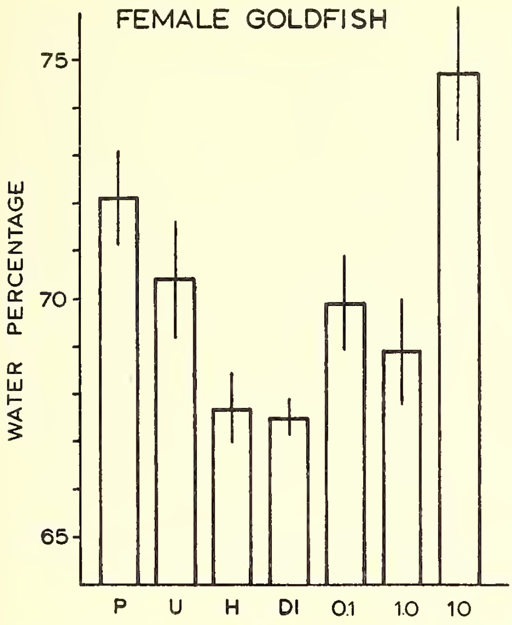
Pituitary extract injection of sufficient dosages (10 and 1.0 μg/gm) significantly raised the ovarian water content over the carrier-injected controls.

Carp Males.—The effect of bringing male carp from the pond into the laboratory and increasing the temperature increased the gonadal water content (3.3%) rather than decreasing it as in the female (2.9%) (Text-figs. 10 & 11).

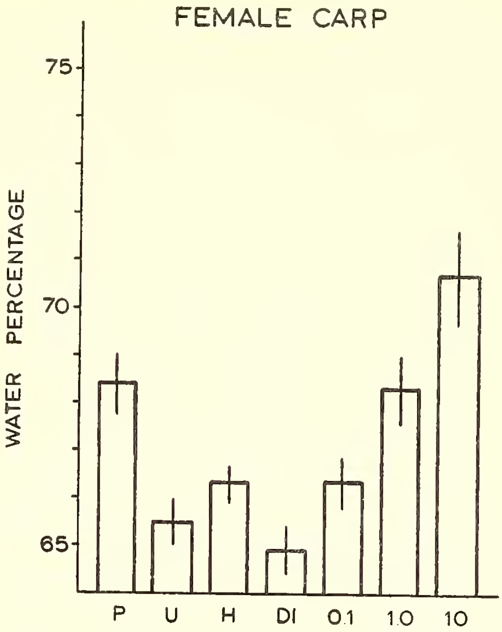
The effect of handling and dummy injecting male carp did not change the gonadal water content measurably over the undisturbed controls. However, the variances in the two former groups were significantly greater (Table III).

Males injected at the medium and higher levels exhibited significantly greater amounts of testicular water than the carrier-injected controls. The lower injection apparently had no measurable effect.

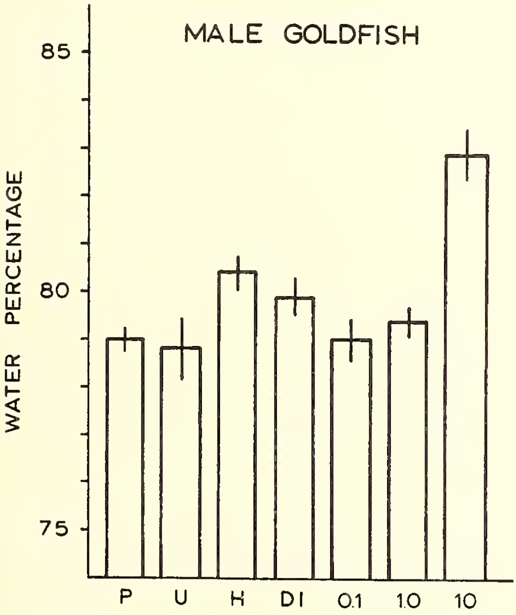
Discussion.—These experiments were designed to determine the effect of handling on the go-



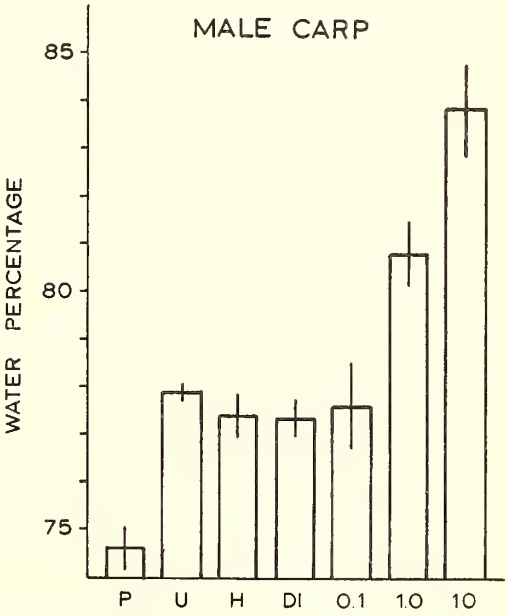
TEXT-FIG. 8. Ovarian water percentages of goldfish from the pond (**P**), "undisturbed" (**U**), experimentally handled (**H**), dummy-injected (**DI**); and injected with pituitary extracts of 0.1, 1.0, and 10 μg/gm body weight respectively. The vertical lines on the bars represent standard errors.



TEXT-FIG. 10. Ovarian water percentages of carp from the pond (**P**), "undisturbed" (**U**), experimentally handled (**H**), dummy-injected (**DI**); and injected with pituitary extracts of 0.1, 1.0, and 10 μg/gm body weight respectively. The vertical lines on the bars represent standard errors.



TEXT-FIG. 9. Testicular water percentages of goldfish from the pond (**P**), "undisturbed" (**U**), experimentally handled (**H**), dummy-injected (**DI**); and injected with pituitary extracts of 0.1, 1.0, and 10 μg/gm body weight respectively. The vertical lines on the bars represent standard errors.



TEXT-FIG. 11. Testicular water percentages of carp from the pond (**P**), "undisturbed" (**U**), experimentally handled (**H**), dummy-injected (**DI**); and injected with pituitary extracts of 0.1, 1.0, and 10 μg/gm body weight respectively. The vertical lines on the bars represent standard errors.

nadal water content of carp and goldfish. The results indicate that the effect of stress induced by experimental handling was opposite in the sexes in both carp and goldfish. The male and female response to stress is probably different only in ovarian and testicular reaction, which is governed by inherent differences in the two types of tissue; probably, stress simply augments the natural process occurring at the time. Prior to this experiment, weekly samples from the pond showed that the relative water content of the gonad was increasing in males and was decreasing in females. We believe the weekly pond samples will demonstrate that the decreasing ovarian water content at this time of the year is the change in the ratio of the cellular water to yolky material. This decrease is followed by an increase in ovarian water content with natural or artificial spawning. We believe the increased ovarian water percentage at spawning is extracellular, homologous to the seminal plasma of males.

Apparently similar handling procedures affected goldfish to a different degree than carp. Being brought into the laboratory and subjected to a temperature change, along with weighing, sexing, fin-clipping and needle puncture, caused a measurable change in the gonadal water content of goldfish. Simply bringing them into the laboratory and subjecting them to the same temperature change did not. Contrary to the observed effects in goldfish, the significant gonadal changes in carp occurred between the pond fish and the undisturbed fish rather than the undisturbed and handled or dummy-injected ones. Evidently, the effect of stress from the temperature increase did not play a major role in producing gonadal water fluctuations in goldfish.

In carp, the effects induced by a temperature increase and those induced by handling are inseparable under the conditions of the experiment, but it is believed that the stress of the laboratory rather than the stress from a temperature increase was the major factor. Certainly under laboratory conditions carp exhibit much "wilder" behavior than goldfish.

Temperature changes, however, should not be eliminated entirely as a stress factor since Hoar & Cottle (1952) reported that the water content of muscle and liver of goldfish varied directly with the change in acclimatization.

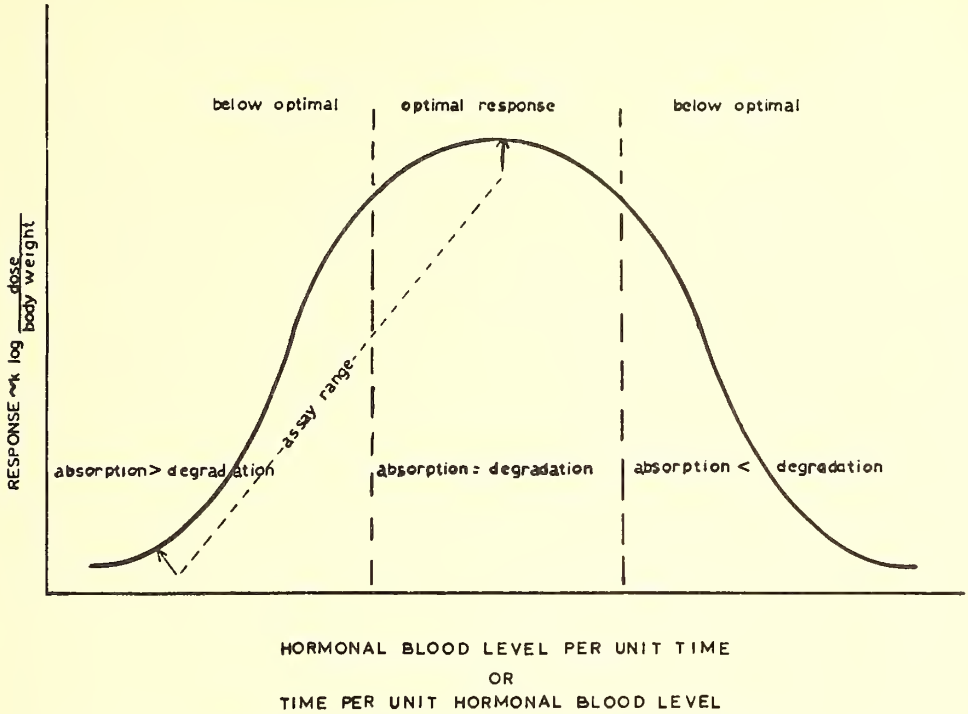
There is no apparent reason why carrier-injected female carp had a significantly ($p=.025$) lower ovarian water content than the handled controls, although male carp and both sexes of goldfish showed no measurable change between the gonads of the two control groups. This suggested that female carp may be more susceptible to stress than males.

There is good evidence that in fishes as in higher vertebrates, adrenocortical hormones are involved in stress, the regulation of metabolism, and the hydromineral balance (see reviews by Pickford & Atz, 1957; Fontaine, 1956; Chester Jones, Phillips & Holmes, 1959; Ball, 1960; and Chester Jones & Phillips, 1960). Both glucocorticoids and mineralocorticoids are known in fishes. Cortisol and cortisone have been found in the blood of carp; although Chester Jones & Phillips suggest it to be universally present in fish, to date aldosterone has been identified only in salmon plasma. Hatey (1958), as reported by Chester Jones & Phillips (1960), was able to detect different levels of 17-hydroxycorticosteroid in the plasma of "unstressed" carp, slightly stressed carp and carp stressed by forced swimming. That such a system could be operating in the regulation of gonadal water awaits future experimentation, and the establishment of the physiological roles of the corticosteroids in fish.

DISCUSSION

The purpose of this study was to pursue the role of the gonadotropins in the reproductive cycle of fish, particularly with respect to water (and electrolyte) movement in the gonads. The data collected established that the testes undergo changes in water content in a way similar to the changes observed in the seminal plasma (Clemens & Grant, 1964). We suspect the same processes are involved in both changes and the observations on the gonads merely reflect the changes of the seminal plasma. The anatomy of the gonads is consistent with this view, since the semen is stored in the seminiferous tubules which are an integral part of the testes. In the female, an ovarian increase in water at the time of ovulation has been established. In both male and female goldfish the gonadal water changes were observed in nature and can be induced by the injection of water extracts of whole ground acetone-dried pituitary of carp. We are confident, then, that the observed responses, gonadal hydration changes in both sexes, play an important role in fish reproduction—in the male it is the preparation of the semen for discharge and in the female it is ovulation.

Since bioassays usually involve placing a given quantity of hormone in an animal, we can assume that as the hormone is absorbed there is a rise in the blood level to a maximum with a subsequent fall (Text-fig. 12). We can relate the rise in the level to hormone absorption and the fall in the level to hormone degradation and refer to three distinct stages, (1) absorption rate greater than degradation rate, (2) absorption equal to degradation, and (3) absorption less than degrad-

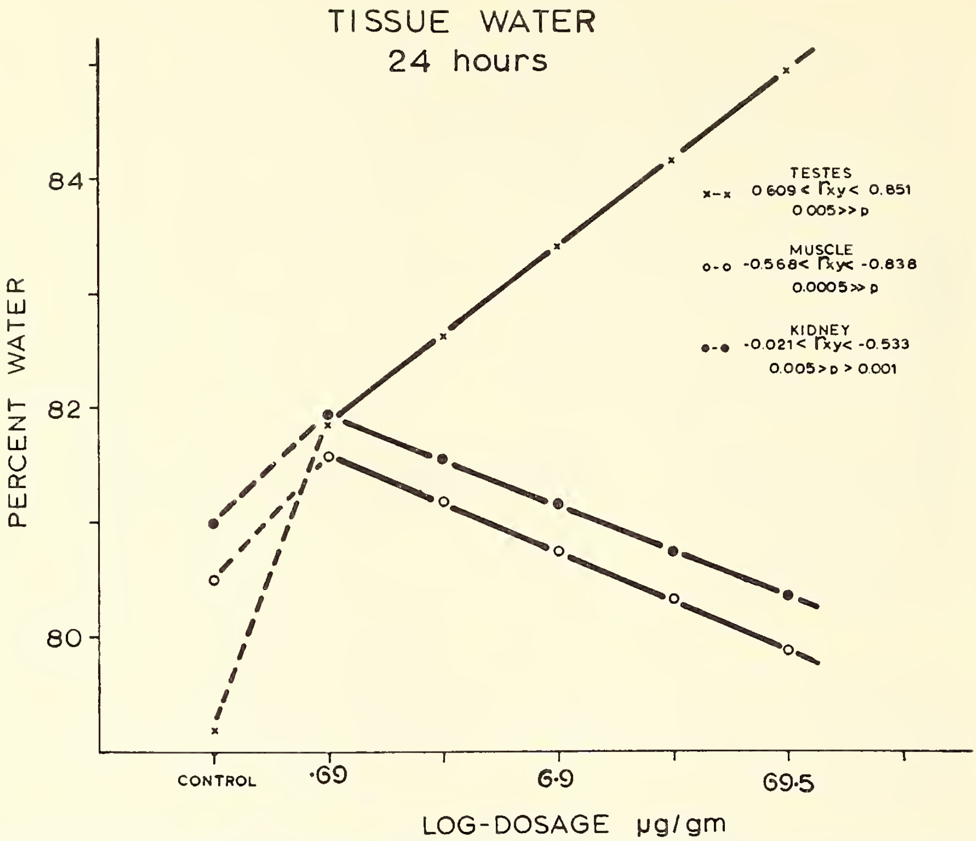


TEXT-FIG. 12. Theoretical curve after hormonal injection.

ation. We think the seminal fluidity response with respect to time (Text-fig. 1) reflects in general the blood level of the hormone. We realize that the graphic figuration of the hormone level may not coincide with that of the response level but until this relationship is learned, one might be used as the approximation of the other.

The role of the endocrines in water and electrolyte metabolism in fish has received considerable attention and in the reviews of Fontaine (1956), Pickford & Atz (1957), Black (1957), and Chester Jones, Phillips & Bellamy (1962), to mention a few, gonadal hydration is an unknown subject. Likewise, in the reviews of fish gonadotropins (Pickford & Atz, 1957; Hoar, 1957; Pickford, 1959; Ball, 1960; and Marshall, 1960), only vague or incidental references are made to this subject. Other organs have received attention. For this reason, the water content of the kidney, muscle and brain of carp (same fish as reported pages 194 and 196) were studied with respect to gonadal hydration. Changes occurred in these organs, with the exception of the brain in which there apparently is no response. The changes that occurred in the muscle and the kidney varied with the size of the injection. Twenty-four hours after injection, the water of the muscle and the kidney showed a decrease with increasing injection (Text-fig. 13), a response opposite to the gonads. The eight-hour observations showed that the kidney and the muscle were dehydrating along with the gonad (Text-fig. 14). Thus, hydration responses in comparison to the controls were much greater for the gonad than those of the kidney and the muscle. The mechanism for hydration was observed to involve the gonad in a much wider range of hormonal levels than the muscle and kidney. Two of several lines of apparent interpretations might be mentioned here. Muscle changes appear to be a transient, passive response, a temporary shift in the water compartments resulting from the interplay of the kidney action and the circulatory system, or even from osmotic changes in the blood alone. Although this shift in water compartments involves the gonads and other tissues, it appears selective for the gonads, possibly because the gonads serve as an open-end compartment allowing a considerable differential between the inward and outward (testes to blood) movement of water. The second possibility is simply that hormonal impurities in the injection caused side effects or that other endocrine systems were brought into play as a result of the testicular response (steroids, etc.).

In experiments with goldfish, other organs, in

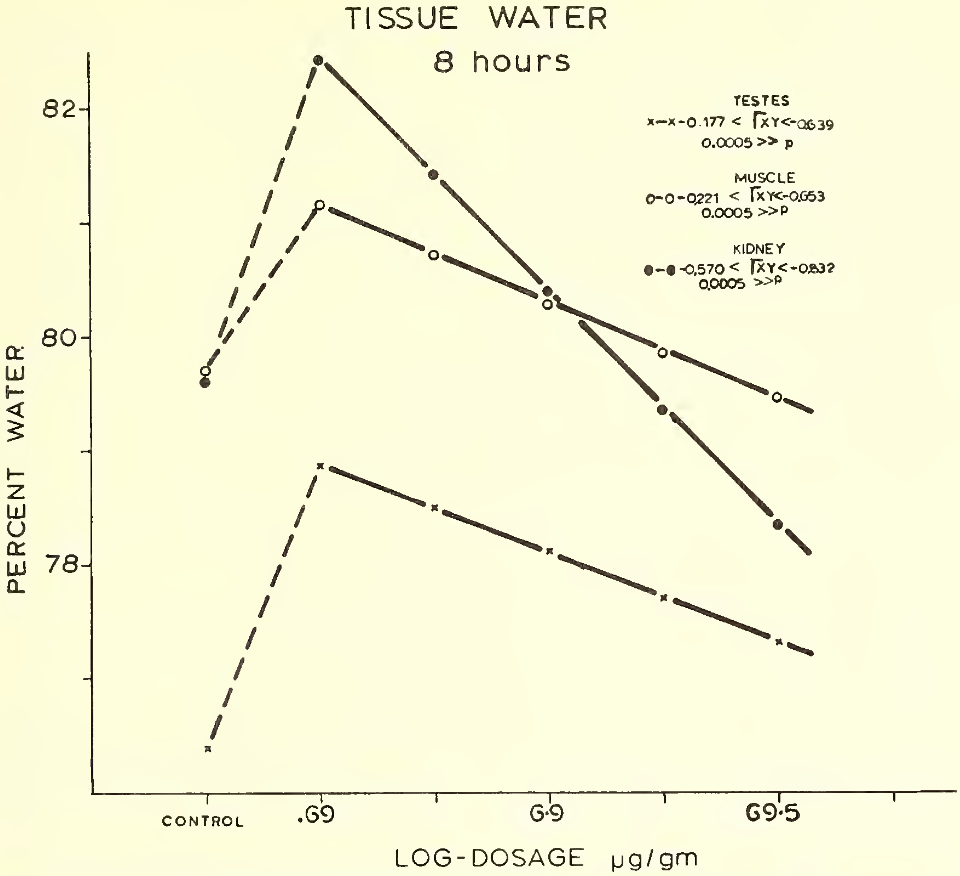


TEXT-FIG 13. Hydration of testes muscle and kidney of carp 24 hours after homoplastic injections of pituitary extract.

addition to the kidney and muscle, were shown to undergo hydration changes in proportion to the injected dosage. The opercular bone either with or without skin (Text-fig. 15) produced a more marked change than either the muscle or the kidney, and the changes corresponded closely to those observed in the testes. The blood of male carp changed with injections of pituitary extract, since the red blood count, the haematocrit and the haemoglobin were observed to rise with concomitant rises in gonadal water (Table IV).

These data on hydration changes in other parts of the body are presented as concomitant events and in a problem as complex as this they are presented only to show that gonadal hydration appears to be rather large in relation to responses in other organs. This is taken to mean that a major response is in the gonads. If the response of the gonads is one associated with spawning, as it is believed to be, then the fish at the peak of the response are expected to be in a highly active physiological state, the presumptive processes involving a number of endocrines, water and electrolyte movement. It is assumed that we are observing this in general response in the body and the gonadotropin(s) involved seems certain to affect the intake of water and electrolytes into the gonad and may do the same on a smaller scale for other body organs and/or may excite other endocrines we believe to be associated with water and electrolyte metabolism. At any rate, we believe that in a problem this complex some common denominator measuring the activity of the various endocrines at the time of spawning is needed and that water and electrolytes may serve this need. Another need is the culture of gonadal tissue with the production of normal cells so that the responses of various hormones can be observed under isolated conditions.

Mammalian vasopressin, oxytocin, pituitrin, LH, FSH, ACTH, STH, TSH and prolactin, though they were administered through a wide range of dosages, showed much smaller and less consistent changes in the testicular water that were unlike the 24-hour observations resulting from even small injections of carp pituitary. This means that goldfish were refractory to mammalian hormones at 24 hours, but these hormones



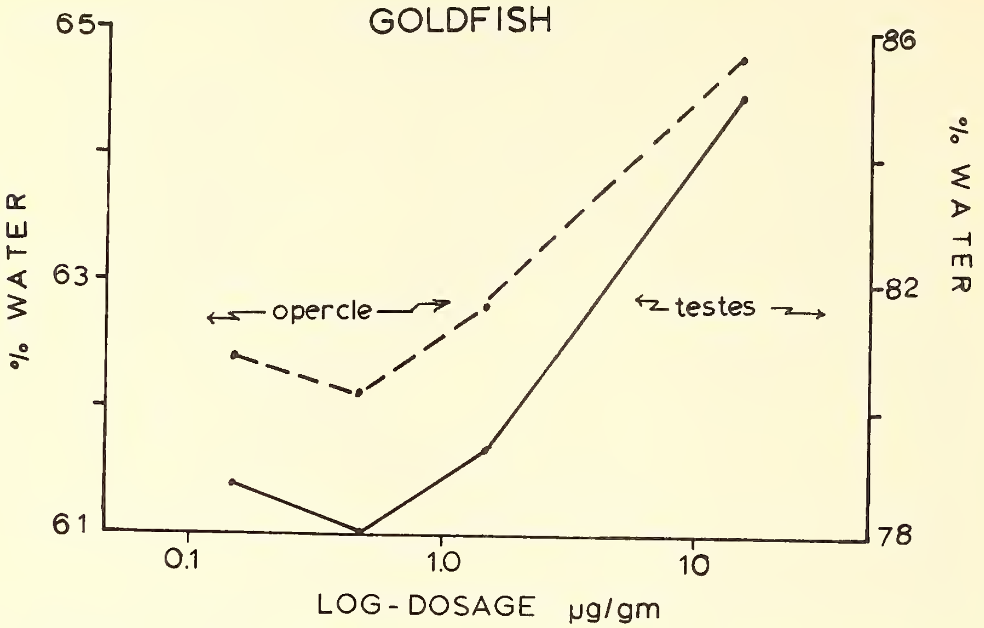
Text-fig. 14. Hydration of testes, muscle and kidney of carp 8 hours after homoplastic injections of pituitary extract.

might have an effect on gonadal hydration at some other period of observation.

The reported chemical properties (Clemens, *et al.*, 1963) suggest the active principle is more like the gonadotropins than any of the other mammalian pituitary hormones. The gonadal response pattern is the basis for purification studies of the active principle now in progress in this laboratory.

The literature contains a number of instances where gonadotropins are suggested or reported as having a role in water and electrolyte metabolism. Pickford & Atz (1957) point out that a number of papers suggest (1) the pituitary of fishes is in some way concerned with calcium metabolism and that the gonadotropins may have an effect on the salt content of the blood of fishes, (2) the ability of fish to withstand or even seek changes in the salinity or osmotic pressure of the water surrounding them needs to be considered in the light of the drastically different environments encountered by anadromous and catadromous fishes on their spawning migrations. In birds, Riddle & Dotti (1945) showed that the pituitary hormone which increases plasma calcium in doves and pigeons is a gonadotropin. Breneman & Zeller (1961) and others show that P^{32} uptake in the testes of the chick is increased after injections of LH and FSH secured from the National Institute of Health. Radioactive phosphorus is taken up in the testes of goldfish in proportion to the dosage of carp pituitary extract. Rugh (1939) stimulated spermiation with hypophysial implantations and described the process in male toads. No study has been found that deals directly with gonadal hydration in fish, although there have been some indications of increased flow of semen after injections of pituitary materials (Clemens & Grant, 1964). Clemens & Sneed (1962) used an increase in seminal fluidity to indicate the presence or absence of the principle in fish pituitaries.

Our data show that hydration is an essential part of seminal plasma elaboration in the male



TEXT-FIG. 15. Opercular bone (with skin) and testicular hydration of goldfish 24 hours after injections with carp pituitary extract.

and ovulation in the female and is hormonally controlled. The hormone regulating the hydration process appears to be a gonadotropin and it is a matter of fact to suspect the involvement of electrolytes and other endocrines.

ACKNOWLEDGMENTS

We acknowledge our appreciation to Dr. Arthur Ghent for his statistical advice, Dr. Max Wilcomb for his participation in the P^{32} experiments, Messr. David Adamson and Lloyd Berry for their assistance in collecting the blood data, Mr. Daniel Zellmer for taking part as a National Science Foundation Undergraduate Research Participant in the forced exercise studies, and to Mr. Waynon Johnson who contributed a part of the data incorporated into the standard curve and collected while working on another phase of our gonadotropin studies.

The original stock of carp for our ponds was provided by Mr. O'Reilly Sandoz, Oklahoma Department of Wildlife Conservation, and Mr. James Savage, Resident Engineer, Altus-Lugert Project, U. S. Corps of Army Engineers.

To the many other persons who assisted in a variety of ways, our heartfelt thanks.

SUMMARY

1. The seminal plasma elaboration in prespawning carp and goldfish is reflected by a gonadal hydration, since the seminiferous tubules in these species are an integral part of the testes.

2. With injections of carp pituitary extract, the gonadal hydration undergoes a gradual rise and fall through a period of three days and is believed to approximate the level of the hormone

TABLE IV. A COMPARISON OF THE CHANGES IN THE BLOOD AND GONADS OF MALE CARP AFTER INJECTION OF HOMOPLASTIC PITUITARY EXTRACT

Dosage µg/gm	% H_2O Testes	% H_2O Blood	Hematocrit % cells	Hemoglobin gm/100 ml	Red Blood count millions/mm^3	Fish No.
0	76.1	85.3	35.0	8.33	1.52	3
1	78.2	82.5	34.3	8.08	1.84	3
10	83.3	79.5	38.3	8.95	1.96	3
100	84.9	84.0	39.2	9.88	2.03	3
316	85.3	85.1	36.8	8.88	2.08	3

in the blood. Twenty-four-hour responses provide an ideal bioassay for following the hormones in chemical isolation procedures.

3. Responses can be obtained throughout the year, although the periods of greatest response appeared during May, June and October.

4. If gonadal hydration is hormonally regulated, then its study in control fish and in pondfish (fish in nature) should reflect the endogenous hormone level.

5. The control fish showed a greater seasonal variation than the injected fish and a reduction of gonadal water below the pondfish during the period from April through June which appeared to be traced to insufficient food, although the feeding rate was the same throughout the year. Goldfish apparently need more food during the prespawning period. Gonadal water of starved fish decreased from 81.9 to 72.9% in 23 days.

6. The effects of bringing male goldfish from the pond into the laboratory and raising the water temperature alone caused a slight decrease in gonadal water which was not statistically significant, while the effects of bringing the fish into the laboratory and raising the water temperature along with the handling involved in their weighing, sexing, fin-clipping and needle puncture caused a 1.6% rise in the gonadal water content ($p=.05$).

7. Gonadal hydration of male goldfish in the pond was relatively high during the prespawning and spawning periods, and during the late summer and early fall.

8. It appeared that endogenous blood levels could not be altered significantly during March by raising the temperature from 3° C to 22° C, although marked changes in gonadal hydration were induced with injections at temperatures of 16.0 and 22.0° C and little or no response was observed at 3.0 and 10.0° C. These observations suggest that temperature increases in nature may not excite hormonal release but merely enhance the effect of the release.

9. Gonadal fat was carefully dissected away from any portion of the gonad used in water determinations, since fat could substantially reduce the water content.

10. The gonadal water of female goldfish increases when injected with the same pituitary materials as the males. It appears that the increase of water is associated with ovulation, since the average ovarian content of females that had been induced to ovulate with injections of pituitary materials was 75.5%, and ovulating females captured in the pond in the act of spawning was 81.3% while non-spawning females had an ovarian water content of 65.2%.

11. The effect of stress induced by experimental handling was opposite in the sexes in both carp and goldfish. In females a small but significant decrease in ovarian water was observed while in males the observed response was a small but significant increase in testicular water.

12. Apparently similar handling procedures affected goldfish to a different degree than carp. Being brought into the laboratory and subjected to a temperature change, along with weighing, sexing, fin-clipping and needle puncture, caused a measurable change in the water content of goldfish. Simply bringing them into the laboratory and subjecting them to the same temperature change did not. Contrary to the observed effects in goldfish, the significant gonadal changes in carp occurred between the pond fish and the undisturbed fish rather than the undisturbed and the handled or dummy-injected ones. Evidently temperature did not play a major role in producing gonadal water fluctuations in goldfish but the effects of temperature increase and those of handling were inseparable in carp under the conditions of the experiment.

13. Hydration changes concomitant with those of the gonads were observed in the muscle, kidney, bone and blood but were relatively small in relation to those of the gonad.

14. The hormone regulating the hydration process appears to be a gonadotropin and it is a matter of fact to suspect the involvement of electrolytes and other endocrines.

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13

Experimental Hybridization of the Green Treefrog *Hyla cinerea* Schneider (Hylidae)

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Plates I & II

EXTERNAL fertilization in most frogs and toads permits the investigator to attempt fertilization between selected anuran gametes and to observe subsequent stages of development. This relatively easy access to the gametes of most anurans has been used to advantage in studies of amphibian genetics (Pyburn, 1961), embryology (Cusimano, Fagone & Reverberi, 1962), cytology (Moore, 1961), evolution (A. P. Blair, 1941; Mecham, 1961). Numerous other pertinent studies could be cited. The objective in making the series of crosses described in this account was to determine if the evidence obtained from experimental hybridization supports judgements of relationship between these hylid species that have been made in the absence of such information.

Gross morphology has long been the major, if not single, source of evidence underlying most judgements of anuran relationships but with the availability and the refinement of techniques which permit the analysis and comparison of amphibian breeding calls (W. F. Blair, 1958c), seroprotein patterns (Bertini & Cei, 1961), chromosomes (Sanders & Cross, 1964), and others, such sources of information can no longer be ignored when the most convincing statement of anuran species relationships is desired. One additional source of information is experimental hybridization and if it be assumed that closely related species are more likely to form viable hybrids between them when experimentally crossed than they are when crossed to more distantly related species, then it seems reasonable to attempt experimental hybridization whenever possible and to make available this evidence so that it might be considered in evaluating relationships between the species crossed. Information from experimental hybridization has proved useful in phylogenetic studies of the genus *Bufo* (W. F. Blair, 1963) but a comparable quantity of data has been lacking for members of the genus *Hyla.*

MATERIALS AND METHODS

All eggs used in these crosses were produced by two female *Hyla cinerea.* These females are distinguished by letter in Tables 1 and 2. Fertilization of *H. cinerea* eggs was attempted using sperm suspensions from *H. cinerea, H. crucifer, H. versicolor, Acris crepitans* and *Pseudacris clarki.*

The method of experimental hybridization is similar to those described by Pyburn & Kennedy (1960). In brief, eggs of ovulating females were introduced into separate sperm suspensions prepared from the testes of individual males. Embryos were maintained in enamel pans containing water. Larvae were transferred to 5-gallon aquaria. At metamorphosis the young frogs were placed in laboratory cages containing a basal layer of damp soil. They were fed laboratory-raised *Drosophila, Tenebrio* larvae and small insects caught by sweeping with insect nets. All frogs used in this study, except *Hyla crucifer,* were collected by me.

RESULTS

Results of crosses with two female *H. cinerea* (A, B) are summarized in Tables 1, 2. The latest stage of development attained by the offspring of the following combinations has been listed by Pyburn & Kennedy (1961).

♀ *Hyla cinerea* (A) × ♂ *Hyla versicolor.* Most eggs cleaved but failed in gastrulation. Twenty-six larvae hatched beginning on the third day

Table 1. Crosses between a female *Hyla cinerea* (A) from Fort Bend County, Texas, and males of the species in the left hand column. Localities from which the males were collected are listed beneath each. Percentages of hatch and metamorphosis are based on number of cleaved eggs.

Male	Eggs	Cleavage Number (%)	Hatch %	Metamorphosis %	Development
Hyla versicolor Fort Bend Co., Texas	485	462 (95.7)	5.6	1.5	Adult
Acris crepitans San Patricio Co., Texas	156	130 (83.3)	49.2	0.0	Tadpole
Hyla cinerea (control) Fort Bend Co., Texas	157	157 (100.0)	96.8	35.1	Adult

after fertilization. Two hybrids lacked the left eye. Three hybrids could not close the mouth. The lower jaw was permanently deflected so that the tongue was always visible (Plate I). The remaining, apparently normal, hybrid was inadvertently crushed by the lid of the cage.

Shortly after metamorphosis, the young frogs changed from a pale green to gray with dark dorsal markings similar to the male parent. The white subocular spot characteristic of *H. versicolor* was evident in all frogs obtained from this cross. All of the hybrids except two individuals with deformed mouths died within 18 days after metamorphosis. One individual died 10 months and 2 days after fertilization and had a snout-vent measurement of 38 mm. after preservation. This frog was always the smaller of the two surviving hybrids and was usually pale green (Plate I B). The other individual (Plate I A) has faint sexual coloration of the throat skin. This hybrid has not called in the laboratory but sometimes chirps when picked up. Even though about the anterior one-half of the lower jaw was permanently deflected in both of these hybrids, they could eat mealworms and small grasshopper nymphs. They could not catch small leafhoppers and small flying insects. Internal structures in these two hybrids were poorly preserved. The gonads could not be located upon dissection and may have been lacking.

Two crosses involving a ♂ *H. cinerea* and ♀ *H. versicolor* have been reported. Tadpoles were obtained from one such combination, but they did not transform and all of the tadpoles died within a few days after hatching (Pyburn & Kennedy, 1960). One young frog was obtained from this combination by Littlejohn (1961) but this frog died about two weeks after transformation. Both of these crosses of ♂ *H. cinerea* × ♀ *H. versicolor* resulted in a lower percentage of development than the reciprocal cross.

♀ *Hyla cinerea* (A) × ♂ *Acris crepitans.* All of the surviving embryos were in gastrula one day after fertilization but gross abnormalities in gastrulation hindered development. Many embryos could not incorporate the abnormally large yolk plugs and did not show subsequent development. Others had greatly distended bellies. The first individual hatched five days after fertilization.

No frogs have been obtained from crosses of *Acris crepitans* with any U. S. member of the genus *Hyla.*

♀ *Hyla cinerea* (A) × ♂ *Hyla cinerea* (Control). All of the 157 eggs cleaved and no abnormalities were observed in the early developmental stages. One hundred fifty-two young tadpoles were obtained of which 55 metamorphosed. Mortality in the tadpole stage was probably due to space limitations. There were no external abnormalities among the young frogs. All were pale green with a distinct lateral white stripe that was variable in width and length. In some individuals, the white stripe had

Table 2. Crosses between a female *Hyla cinerea* (B) from Fort Bend County, Texas, and males of the species in the left hand column. Localities from which the males were collected are listed beneath each. Percentages of hatch and metamorphosis are based on number of cleaved eggs.

Male	Eggs	Cleavage Number (%)	Hatch %	Metamorphosis %	Development
Hyla crucifer Wyoming Co., Penn.	197	158 (80.0)	2.6	–	Tadpole
Pseudacris clarki Harris Co., Texas	260	156 (60.0)	4.5	–	Tadpole

a narrow black border (Plate II A). The golden chromatophores evident as irregular dots on the dorsum of adult *H. cinerea* were not evident in the frogs immediately after metamorphosis but appeared as the frogs increased in size.

An F_1 male of 43 mm. snout-vent length called 58 days after fertilization. The air temperature in the laboratory was 24.4 C. This was the first call heard from these frogs, although various males called at irregular intervals thereafter. The call sounded like those of typical wild male *cinerea.* Twenty-four frogs from the control cross were preserved. The range in snout-vent length is 30-48 mm., with a mean of 36 mm. There were 18 males and 6 females. The observed sex ratio differs significantly from a theoretical 1:1 ratio beyond the 5% level of probability ($\chi^2 = 5.04$). If the expected 1:1 sex ratio is correct, then either fewer females than males survived or fewer females than males were produced from this cross. It is also possible that the 24 frogs do not represent a random sample.

Microscopic sections of the ovaries of one female showed that normal oogenesis was in progress but that no mature eggs had been formed. This female was preserved 128 days after fertilization and her snout-vent length was 35 mm. Histological preparations were not necessary to determine the stage of oogenesis in three females because their ovaries contained large masses of well-developed eggs. Snout-vent lengths of these females were 40, 42 and 45 mm. Their oviducts were evident upon dissection as slightly convoluted tubes. All three females were preserved and measured 500 days after fertilization. The ovaries of two other females, 33 and 37 mm. snout-vent lengths, did not contain eggs that were visible upon dissection. Poor preservation made histological study of the ovaries not feasible. There is no evidence to indicate that normal oogenesis was not occurring in each of these females.

Microscopic sections of the testes of 17 males were examined. The testes of one frog were not examined histologically because of poor preservation. Spermatozoa were present in the testes of all but one of 17 control males. The mean snout-vent length of these individuals is 41.4 mm. (30-48 mm. range). The frog whose testes did not possess spermatozoa has a snout-vent length of 39 mm., which is well within the range of sexual maturity indicated by the control males. The left testis of the sterile frog was the smallest of the group measured, having a length of 3.0 mm. and a width of 1.0 mm. Measurements of snout-vent length and testes length and width were made on 14 apparently fertile F_1 controls. The mean snout-vent length is 41.5 mm. (30-48 mm. range). The mean length of the left testis of these 14 males is 4.5 mm. (3.3-6.0 mm. range); the mean width is 2.0 mm. (1.7-2.6 mm. range). The testes of two additional frogs were not measured but contained spermatozoa. The only abnormality noted upon dissection of the controls was that one frog had a greatly distended urinary bladder which contained a copious white mucus.

♀ *Hyla cinerea* (B) × ♂ *Hyla crucifer.* Eighty percent. of the 197 eggs cleaved and developed to blastula without major abnormalities, but only 40% attained gastrula (Table 2). Difficulties in gastrulation resulted in most of the mortality, and only 10% developed into neurula. Four larvae hatched about three days after fertilization but one died shortly after hatching. Two of the young tadpoles died within 10 days after fertilization. The remaining tadpole was notably abnormal. The tail was deflected and the body was larger than normal in proportion to the tail. These abnormalities did not seem to interfere with feeding. Front limbs had not appeared when this individual died 72 days after fertilization. The labial tooth formula is $\frac{2}{2}$. The upper horny beak is denticulate. A lower beak is not present.

♀ *Hyla cinerea* (B) × ♂ *Pseudacris clarki.* One hundred thirty embryos developed into gastrulae, but only 19 embryos attained the stage of tail bud. Seven larvae hatched beginning on the third day after fertilization. Although the young tadpoles were very active and did not appear abnormal, all but three died within five days after hatching. The last surviving individual died seven days after hatching. One larva hatched from the reciprocal combination, ♀ *Pseudacris clarki* × ♂ *Hyla cinerea;* all embryos were twisted and the one that hatched died soon afterward (Littlejohn, 1961).

Discussion

All of the crosses were attempted between members of the family Hylidae. Thus the species crossed share some common morphology at least at the familial level. Two of the crosses which failed to produce F_1 frogs were intergeneric combinations of *Hyla cinerea* with *Acris crepitans* and *Pseudacris clarki.* The morphology of *A. crepitans* and *P. clarki* would not appear to support an argument for a close relationship to the larger and arboreal *Hyla cinerea* (Wright & Wright, 1949). The breeding calls of these species have been analyzed by W. F. Blair (1958a, b, c) and do not suggest a close relationship. It can tentatively be concluded from the single cross of ♀ *H. cinerea* × ♂ *Pseudacris clarki* that this combination shows reduced survival and that the tadpole is not capable of metamorphosing. Genetic incompatibility is expressed in the stages preceding metamorphosis and largely in the pretadpole stage. I am fully cognizant that no control cross was attempted for this experimental cross and that the results of single crosses must be interpreted with caution. The tadpoles obtained from the ♀ *Hyla cinerea* × ♂ *Acris crepitans* failed to metamorphose. The occurrence of a sterile adult *H. cinerea* male in the F_1 controls (Table 1) points out the advisability of checking suspensions in which the testes of wild-caught males have been macerated for the presence of sperm capable of fertilization.

Another combination that did not produce adults is that of ♀ *H. cinerea* × ♂ *H. crucifer*. *Hyla crucifer* does not appear to be closely related to *H. cinerea* on the basis of morphological evidence. According to Lynch (1962) the presence of the ilial shaft ridge in *Hyla crucifer*, *Acris crepitans* and *Acris gryllus* may indicate that *H. crucifer* is more closely related to *Acris* than *Hyla*. The ilial shaft ridge is not present in the species of *Hyla*, which included *H. cinerea*, examined by Lynch. The number of maxillary teeth is considerably greater in *Hyla cinerea* than in *Acris crepitans*, *Pseudacris clarki* and *Hyla crucifer* (Goin, 1958). The call of *H. crucifer* does not fit closely to any U. S. *Hyla* but one type of *H. crucifer* call shows resemblances to calls of *Pseudacris streckeri* and *P. ornata* (W. F. Blair, 1958a). Hybrid inviability in the combination of *Hyla cinerea* × *Hyla crucifer* was expressed in the stages preceding metamorphosis, but this conclusion is uncertain because no control cross was made. However, the available information from this cross and other sources does not indicate a close relationship of *H. crucifer* with *H. cinerea*.

Differences in chromosome number could contribute to hybrid inviability and the chromosomes of several of the frogs hybridized have been studied. According to Bushnell, Bushnell & Parker (1939), 12 is the haploid chromosome number of *H. versicolor* and *H. cinerea*. The chromosomes of these species are similar in that the haploid complement consists of seven large and five smaller chromosomes. Adults were produced in the hybrid combination of *H. versicolor* with female *H. cinerea*. Difference in chromosome number is a potential source of hybrid inviability in the combination with *Acris crepitans* in which the haploid number is 11 (Bushnell, Bushnell & Parker, 1939), and with *H. crucifer* in which the haploid number is probably 13 (Witschi, 1933).

Results of the combination between *Hyla cinerea* and *Hyla versicolor* suggest a greater degree of genetic compatibility between these two species than was suspected solely on the basis of their external morphology. The percentage of hatch and metamorphosis from this combination is considerably lower than in the controls. Even though adults were obtained, development from this cross was limited and a reduction in interfertility is indicated. The calls of the two species show sufficient divergence to place them in different species groups. This and other evidence suggests that the closer affinities of *Hyla cinerea* lie within the *cinerea* species group (W. F. Blair, 1958a). Natural hybrids between *Hyla cinerea* and one member of the *cinerea* species group, *Hyla gratiosa*, have been reported by Bogert (1960) and Mecham (1960). Results of the crosses presented here do not suggest a close relationship between *Hyla cinerea* and *Hyla crucifer*, *Hyla versicolor*, *Pseudacris clarki* or *Acris crepitans*. Additional crosses are needed before the full potential of genetic compatibility between these species can be realized.

Statements of the complexities in the use of experimental hybridization (Moore, 1955, 1959; Clark Hubbs, 1963), can be used to avoid inaccuracy in the interpretation of hybridization results. If anuran postmating isolating mechanisms cannot be reinforced by selection (Mecham, 1961), then this complication may not be as pertinent in influencing the degree of crossability as has been thought. Even with other acknowledged complications, the data from experimental hybridization are useful in the determination of anuran relationships (W. F. Blair, 1962) and justify consideration along with the more conventional sources of evidence.

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SUMMARY

Series of experimental crosses were made to determine if the evidence from experimental hybridization supports current judgements of relationships between the species crossed. Four hybrid and one control combination were made using eggs from two *Hyla cinerea*. If the latest stage of development be used as a crude expres-

sion of genetic compatibility, then the results of hybridization tests generally support judgements of relationship between the species crossed but the evidence presented here must be interpreted with caution, for problems inherent in experimental hybridization, such as gynogenesis and others (see Clark Hubbs, 1963), were not clearly excluded.

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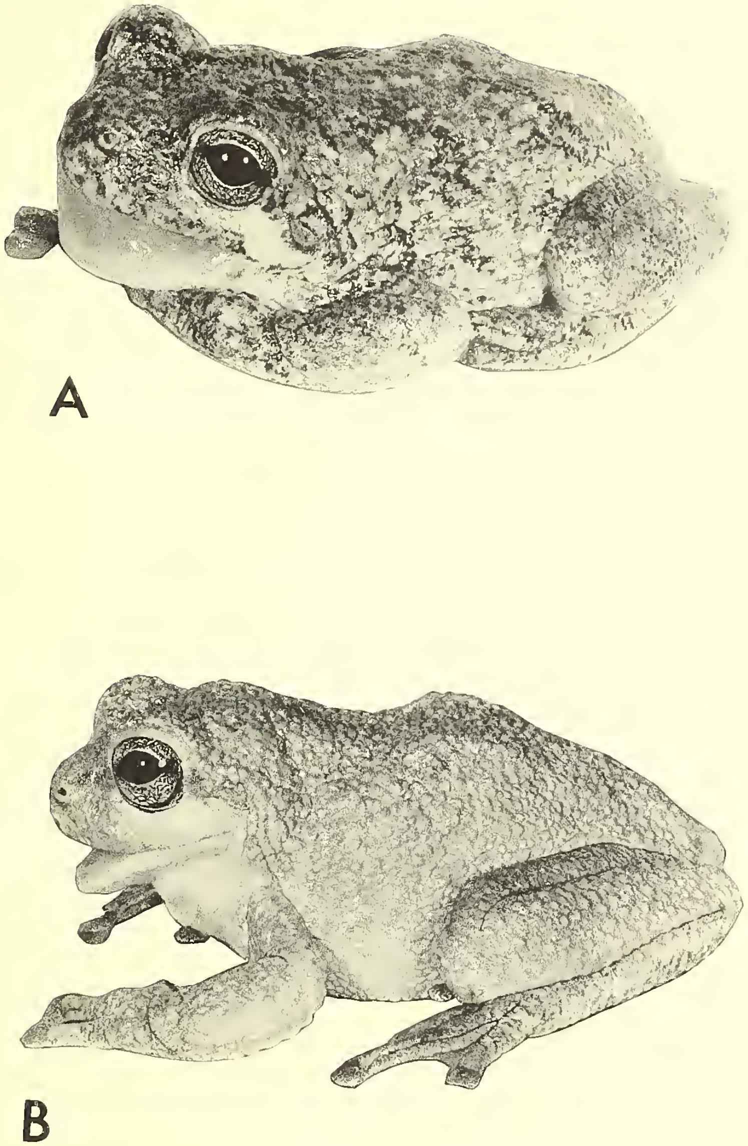
EXPLANATION OF THE PLATES

Plate I

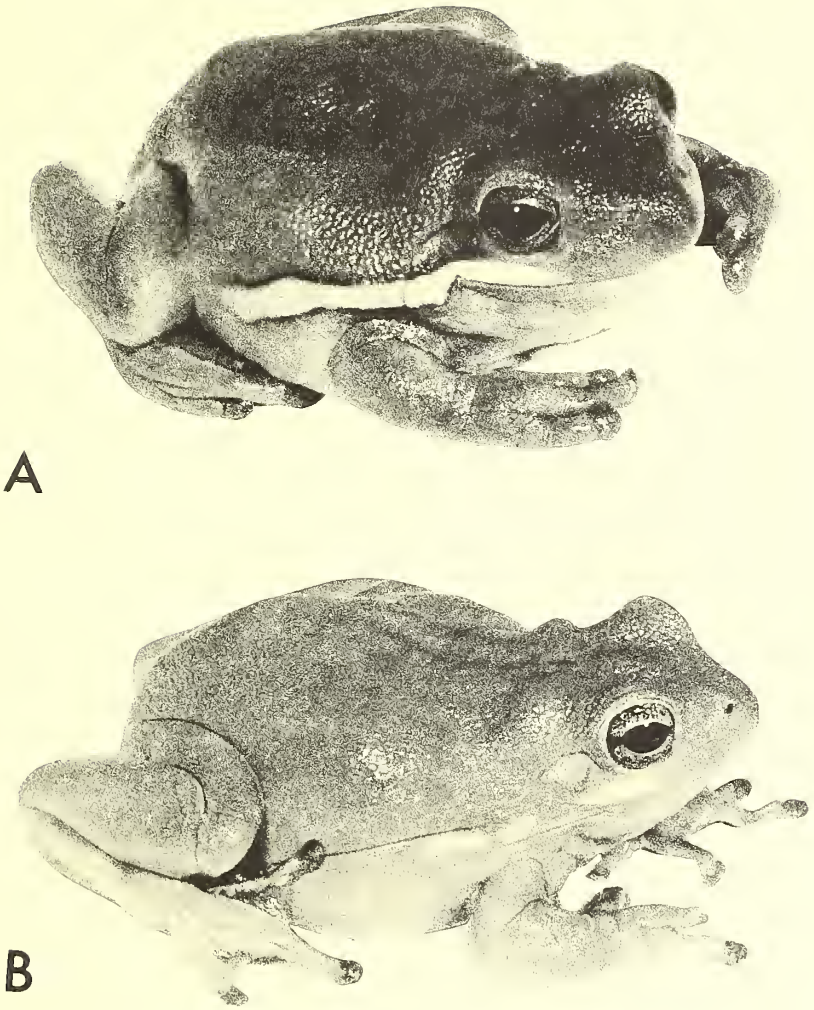
Fig. 1. **A.** F_1 ♂ (♀ *Hyla cinerea* (A) × ♂ *Hyla versicolor).* **B.** Same cross, different individual. Neither hybrid could close its mouth. The white subocular spot characteristic of *versicolor* is shown.

Plate II

Fig. 2. F_1 (♀ *Hyla cinerea* (A) × ♂ *Hyla cinerea*) controls. Variability of the lateral stripe is shown. **A.** 47 mm. snout-vent frog was very dark green when photographed. **B.** 45 mm. snout-vent frog was very light green when photographed.



EXPERIMENTAL HYBRIDIZATION OF THE GREEN TREEFROG
HYLA CINEREA SCHNEIDER (HYLIDAE)



EXPERIMENTAL HYBRIDIZATION OF THE GREEN TREEFROG
HYLA CINEREA SCHNEIDER (HYLIDAE)

14

Studies on the Pituitary Gland of *Xiphophorus maculatus* (the Platyfish)[1]

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(Plates I-V; Text-figure 1)

INTRODUCTION

IT is generally accepted that histological and cytological variations in the hypophysis reflect the physiological state of the individual. Although a large body of information relating pituitary gland structure to function in mammals is available, comparatively little is known about the pituitary gland in fishes under different physiological conditions. This may be attributed, in part, to the difficulty of handling and studying these tiny glands with their extremely small cells. General pituitary morphology and histology have been described in many fishes, but there is only a modicum of information dealing with detailed cytological analysis. The more refined staining methods that have been applied to mammals *(e.g.,* Halmi, 1950, 1952; Hildebrand, Rennels & Finerty, 1957; Purves & Griesbach, 1951 a, b, c) have been utilized in studies of fish pituitaries in only a few instances *(e.g.,* Atz, 1953; Barrington & Matty, 1955; Sokol, 1961; Van Mullem, 1958).

The platyfish *(Xiphophorus maculatus)* of the Genetics Laboratory of the New York Aquarium, New York Zoological Society, present a unique opportunity to analyze the relation between the endocrine system and normal and abnormal development. These valuable experimental animals of known genetic constitution can be obtained at any stage of their ontogeny with or without thyroid tumors, melanomas, castration and endocrine transplantation. Essential to any analysis of the functions of the platyfish pituitary is a comprehensive report on its normal histology and cytology. Some published information dealing with the histology and cytology of the platyfish hypophysis comes from investigations dealing mainly with other aspects of fish endocrinology, but because of inadequate techniques these are of limited value.

The objectives of the present investigation, therefore, are to present a comprehensive report on the morphology, histology and cytology of the pituitary gland in normal, sexually mature male and female platyfish. In addition, structural modifications in the pituitaries of platyfish under the following conditions will be presented and analyzed:

(a) Platyfish one to eight weeks of age.
(b) Aging female platyfish.
(c) Gravid female platyfish.
(d) Castrated male platyfish.
(e) Platyfish with thyroid tumors.
(f) Platyfish—swordtail hybrids with melanomas.

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MATERIALS AND METHODS

The 118 platyfish used in this investigation were members of strains (Table I) maintained at the Genetics Laboratory of the New York Zoological Society, under conditions described

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[1] From a dissertation submitted to the faculty of the Graduate School of Arts and Science in partial fulfillment of the requirements for the degree of Doctor of Philosophy at New York University.

Table I. Profile of Platyfish and Hybrids Utilized in This Investigation

Category	Pedigree & Strain	Number Females	Number Males	Age Range (Months)
Sexually Mature	30, 163, C30	16	20	4-12
Immature	C30	13	13	1-8 (weeks)
Old	G981	8	0	28-33
Old Control	G1064, G1137	4	4	5-9
Gravid Female	163, M1, G1064	7	0	5-10
Castrate Male	30×163, C30	0	9	5-8
Heterotopic Thyroid Tumor	995, 996, 987, 163	6	3	12
Melanoma	M1, M2	4	4	5-12
Melanoma Control	M1	5	2	6-8

by Gordon (1950). The inbred strains 30 and 163 originated from specimens collected in the Rio Jamapa, Mexico, in 1939. Strain C30 is essentially identical in its genetic makeup with strain 30 except that one of its X chromosomes has been derived from a Rio Coatzacoalcos strain by introgressive hybridization. The Rio Grijalva strain (G) has been maintained in the laboratory since 1952. Pedigree numbers 995, 996 and 987 are hybrids of Coatzacoalcos platyfish mated to the 30 strain. Strain M1 was created by introducing a *maculatus* chromosome carrying the *Sd* gene into the 3B (Rio Papaloapan) strain of the swordtail, *Xiphophorus hellerii*, by introgressive hybridization. The M2 strain was maintained by mating *X. maculatus*-*X. hellerii* hybrids with each other.

To ascertain the morphological, histological and cytological status of pituitaries in mature male and female fish under non-experimental conditions, four- to twelve-month-old animals were sacrificed at various periods during a complete year. In order to include structural variations associated with aging, immature and aged platyfish were utilized. The immature group comprised individuals one, two, three, four, six and eight weeks postpartum. The sex of these juveniles was determined by sex-linked pigmentary patterns (only females carried the *Sd* gene for dorsal fin spotting). Aged fish were represented by 28- to 33-month-old individuals reared in the laboratory. Male fish castrated at one to four months of age and sacrificed four to seven months later formed the basis for study of the effect of orchidectomy. Fish were used only after gonadectomy had been confirmed by arrested gonopod development and by the absence of testicular tissue at autopsy. Normal mature males of the same age constituted the controls. When certain strains of *Xiphophorus maculatus* are subjected to an iodine-deficient environment by rearing them in distilled water, they develop heterotopic thyroid tumors (Baker, 1958 a, b; Baker, Berg, Gorbman, Nigrelli & Gordon, 1955; Mac Intyre, 1960). In the present investigation, pituitaries were taken only from animals with well-developed heterotopic thyroid tumors. Controls were siblings and mature animals of similar genetic constitution maintained in conditioned aquarium water. Melanotic tumors occur regularly in the hybrid offspring of matings between black-spotted platyfish and swordtails (Gordon, 1951). Only fish with well-defined pigmented tumors were considered, and comparisons were made with non-melanomatous fish from closely related pedigrees and with normal, mature platyfish and swordtails.

Mature specimens for study were taken from Genetics Laboratory aquaria and immediately decapitated just posterior to the operculum. The gill chamber was cleared of tissue and the pituitary exposed by removing the roof of the buccal cavity. The heads were placed in fixing fluid within 60 seconds of decapitation, and after fixation and washing they were partially dehydrated and hardened in 70% and 80% ethanol. The brain and attached hypophysis were then removed from the skull, extrinsic tissue trimmed down and the preparation subjected to final dehydration. Immature specimens (one to eight weeks old) were killed by severing the spinal cord at the base of the skull without separating the head from the body. The snout was trimmed off to the level of the eyes, an incision made into the coelom and the bodies placed in fixing solution. Because extirpation of the pituitary was difficult, juvenile fish were decalcified, after fixation, in a 5% to 6% suspension of disodium ethylenediamine tetracetate (Sequestrene AA of the Alrose Chemical Co., Providence, R.I.) in 10% aqueous formalin. This method satisfactorily softened the bones and did not interfere with subsequent staining.

Consistent and dependable results were obtained from material fixed in Helly's fluid, formol-sublimate (1 part formalin and 9 parts saturated aqueous mercuric chloride) and Elftman's (1957) chrome alum solution. Fixing agents containing acetic acid did not generally produce suitable preparations. They brought about lysis of cells or clumping and shrinkage of cytoplasmic material. All fixatives were prepared immediately prior to use.

Dehydration of all material was accomplished by using the Zirkle normal butyl alcohol series, as described by Krajian (1940, p. 212). Infiltration and subsequent embedding was achieved with 56°-58° C. embedding paraffin. Complete penetration of paraffin into the cavity of immature fish was insured by infiltrating in a vacuum paraffin oven for thirty minutes at 50 to 150 mm. of mercury and sixty minutes at 62° C. at atmospheric pressure. In order to secure proper orientation for sectioning, brains and attached pituitaries and whole juvenile fish were embedded under a dissecting microscope and the cutting plane marked by a fine bristle.

Serial sections, four micra thick, were cut in transverse, frontal and sagittal planes and stained with the following techniques:

(1) The Heidenhain's azan method as adapted by Dawson & Friedgood (1938) with some minor changes, *e.g.*, less time in the orange G-anilin blue counterstain. This method produced excellent and consistent color differentiation of cell types.

(2) Masson's ponceau-acid fuchsin, anilin blue technique (Lillie, 1954, p. 351). A 1 percent. phosphotungstic acid was substituted for phosphomolybdic acid.

(3) The periodic acid-Schiff reaction for glycoprotein as described by Purves & Griesbach (1951a).

(4) The Halmi (1952) modified aldehyde fuchsin technique of Gomori (1950). Elftman's (1959) oxidation procedure was employed as a supplemental step.

Adjacent sections from each of the several representative pituitaries were stained by the above techniques and in this manner a comparative picture of the reaction of cells to a number of techniques was secured. Stained slides were cleared in xylene and mounted in clarite in xylene.

Differential cell counts were determined from four micra, sagittally serially sectioned glands at 1800 × magnification. For each specimen, three sections were utilized: one section from the mid-region and one section from each lateral half of the gland. All cells were classified and counted in a band 58 micra wide that extended from the anterior to the posterior margins of the gland and therefore included the pro-adenohypophysis, meso-adenohypophysis and meta-adenohypophysis. A blue filter (Eastman Kodak H filter #45) was utilized to accentuate the acidophils and a light green filter was employed to differentiate cyanophils from chromophobes.

All dimensions were determined with the aid of an ocular micrometer, and in non-spherical bodies represent the two longest perpendicular axes. Cell and nuclear values represent an average of the measurement for several cells. The terms "length," "width" and "depth," as applied to the total gland, require clarification. Length is the linear measurement along the anterior-posterior axis, width along the dorsal-ventral axis. Depth is the distance between lateral limits and was determined by direct mensuration of transverse sections or by counting the number of the sagittal sections comprising the total gland and multiplying by the thickness of the sections. It was not possible to obtain cell differentials and dimensional determinations for all pituitary glands examined.

Observations

A. Sexually Mature Platyfish

1. Morphology

The hypophysis of *Xiphophorus maculatus* is suspended from the diencephalon by an infundibular stalk immediately posterior to the optic chiasma. There is no sella turcica comparable to that found in mammals; the gland lies just above, but separated from, a slight depression in the parasphenoid bone. The pituitary is ovoid and compressed dorsoventrally. The gland has a mean anterior-posterior length of 472.9 micra, mean width of 178.3 micra and a mean depth of 376.0 micra. Male glands are smaller than those of females, but significantly so for mean depth only (see Table II). In ventral aspect, the gland gradually tapers caudally from the rounded anterior end. The dorsal surface of the pituitary is concave; ventrally it is slightly convex.

The short, thick-walled hollow infundibular stalk forms a lumen which is continuous with the third ventricle. The lumen narrows as it enters

TABLE II. TOTAL GLAND MEASUREMENTS (IN MICRA)

	Length			Width			Depth		
	Mean	S.E.	No. Animals	Mean	S.E.	No. Animals	Mean	S.E.	No. Animals
Mature Female	478.5	20.2	11	189.0	11.5	12	412.0	21.4	12
Mature Male	465.1	12.0	8	164.1	20.4	9	328.0	13.8	9
Mature Male & Female	472.9	12.0	19	178.3	10.1	21	376.0	17.0	21
Castrate Male	576.7	14.2	7	249.5	11.6	6	511.8	25.8	6
Gravid Female	543.2	35.4	4	213.3	24.7	4	475.5	41.5	4
Thyroid Tumor	512.7	17.6	6	223.2	6.9	6	463.1	12.1	7
Tumor Control	433.2	27.9	6	143.2	17.1	6	342.5	19.8	6
Melanoma	457.0	27.6	7	235.7	27.2	7	336.0	22.8	7
Melanoma Control	422.3	13.0	3	180.0	8.4	4	313.3	16.8	3

the mid-dorsal region of the hypophysis and extends into the posterior portion of the gland as the hypophysial recess. The pituitary gland is completely enveloped by a delicate connective tissue capsule.

The hypophysis (Plate I, fig. 1) is composed of four regions: pro-adenohypophysis, meso-adenohypophysis, meta-adenohypophysis and neurohypophysis, according to the terminology proposed by Pickford & Atz (1957). The pro-adenohypophysis, the most anterior portion of the organ, is bounded posteriorly by a very thin connective tissue septum that separates it from the meso-adenohypophysis immediately posterior to it. The meso-adenohypophysis ("Übergangsteil" in the older literature) is contained ventrally by the capsule, posteriorly by the meta-adenohypophysis and dorsally by the neurohypophysis. The meta-adenohypophysis constitutes the posterior part of the gland. Its delineation from the meso-adenohypophysis can be accomplished only by identification of its characteristic cell types. The neurohypophysis occupies the mid-dorsal region of the gland and forms a continuation of the stalk. Projections from it penetrate all regions of the adenohypophysis.

2. Histology and Cytology

The description that follows holds true for both male and female platyfish; no sexual dimorphism in the pituitary is evident.

a. Pro-adenohypophysis

The pro-adenohypophysis may be readily identified by several salient characteristics in addition to its anterior position and relatively large size. It is frequented by vascular channels that are considerably wider than capillaries and may suitably be termed sinusoids. The pro-adenohypophysis is populated by two cell types, carminophilic acidophils, which predominate, and chromophobes. These cells are closely packed and exhibit no particular arrangement except for a distribution of the acidophils according to size. These cells are generally smaller at the anterior portion of the pro-adenohypophysis and larger posteriorly. Morphologically the larger and smaller cells are similar. Chromophobes, in addition to their general dispersion throughout the pro-adenohypophysis, form a peninsula of cells along the anterior side of the connective tissue septum and extend between the lateral portions of the gland. This chromophobic wedge is several cells wide dorsally and tapers as it follows the contour of the posterior boundary of the pro-adenohypophysis ventrally.

Pro-adenohypophysial acidophils are round or oval in shape with eccentric nuclei (Plate I, fig. 2). They range from 6.0 to 8.0 micra in length, 3.8 to 6.3 micra in width and have a mean nuclear length of 4.4 micra. Generally they stain intensely with azocarmine, although in a few instances peripheral cells exhibit an affinity for orange G. With ponceau-acid fuchsin of the Masson method, they are colored red to orange-red. Because the large coarse granules are so closely packed, the cytoplasm oftens appears to be homogeneous rather than granular. Nuclei generally are rounded but may be oval. The disposition of chromatin material shows considerable variation; it may be in fine or coarse granules, centrally clumped, or widely dispersed in the karyoplasm. The chromatin usually stains with acid fuchsin and azocarmine, but it often shows an affinity for anilin blue. A clear area adjacent to the nuclei, presumably the negative image of the Golgi apparatus, is usually in evidence. Nucleoli consistently show an acidophilic (azocarmine and acid fuchsin) staining reaction.

Scattered among the acidophils are the chromophobes (length, 4.0 to 6.0 micra; width, 3.0 to 5.0 micra; mean nuclear length, 3.5 mirca). They are characterized by a modicum of light blue- or gray-staining cytoplasm. A central nucleus, with a heavily stained cyanophilic membrane, encloses scattered chromatin that stains with anilin blue. The wedge-like area, previously described, is comprised of closely packed polymorphic chromophobes.

b. Meso-adenohypophysis

In mid-sagittal section, the meso-adenohypophysis may readily be separated into a dorsal region that contains both acidophils and cyanophils, and a ventral cyanophilic zone. The dorsal region is pervaded by fine projections of neurohypophysial tissue that divide it into irregularly shaped islets of closely packed cyanophils and acidophils (Plate I, fig. 3). The ventral region consists of a less closely packed aggregate of cyanophilic cells. In transverse sections, the dorsal region caps the neurohypophysis and is in turn girdled by the ventral region. Four cell types may be distinguished in the meso-adenohypophysis: acidophils, two types of cyanophils, and chromophobes.

Acidophils (length, 5.0 to 8.7 micra; width, 3.5 to 5.3 micra; nuclear length, 4.1 micra) are generally round or oval although pyramidal-shaped cells are sometimes present. They are coarsely granular, and localized aggregates frequently give the cytoplasm a splotched appearance. With critical differentiation in Heidenhain's azan technique, these granules appear orange. In this method one overstains with azocarmine, differentially decolorizes and then applies the anilin blue-orange G mixture. Those acidophils that have been decolorized (*i.e.*, exhibit a lesser affinity for the azocarmine) will now accept the orange G. For this reason meso-adenohypophysial acidophils occasionally appear tinted with red and infrequently are entirely carmine colored. With Masson's technique, the acidophils are invariably red-orange and cannot be distinguished tinctorially from the acidophils of the pro-adenohypophysis. Round to oval nuclei are displaced peripherally, usually toward one end of the long axis or toward the basal end of the pyramidal-shaped cells. Chromatin material is variable in size and location. It most often accepts anilin blue but may sometimes be colored red or orange with the other acid dyes. Nucleoli are not always demonstrable.

Two classes of cyanophils can be distinguished by their morphology and their position in the meso-adenohypophysis. It is, however, impossible to differentiate between these cyanophils by means of staining intensity with anilin blue. The cyanophils in the central region, that is, the ones closer to the neurohypophysis and in closer relationship to the acidophils, are spherical with a large, round, centrally located nucleus. The diameters of these cyanophils range from 3.0 to 6.0 micra, and they have a mean nuclear length of 3.8 micra. Characteristically they have a uniformly thin rim of finely granular, cyanophilic cytoplasm. The round nucleoli and fine chromatin granules exhibit an affinity for azocarmine. The peripheral cyanophils, by comparison, show little uniformity in shape and vary in size from 5.5 to 9.0 micra in length and 4.8 to 7.5 micra in width. Nuclear structure is also variable. Although their most prevalent form is round or oval, the nuclei may be elongate, constricted or appear slightly twisted. Red nucleoli are found in varying chromatin substance. The cytoplasmic granules of the ventral cyanophils vary considerably from cell to cell and are larger than those of the dorsal cyanophils. Small cytoplasmic vacuoles are often seen. A unique feature of the majority of these cyanophils is the presence of orange or red spherical hyaline droplets in the cytoplasm. These cytoplasmic inclusions vary in size and number per cell.

The chromophobic elements of the meso-adenohypophysis are not as prevalent as their counterparts in the pro-adenohypophysis, but they are very similar in structure.

c. Meta-adenohypophysis

The meta-adenohypophysis is the most posterior region of the platyfish pituitary, and it is extremely variable in extent and cellular arrangement. It also encompasses more neurohypophysial tissue than any other region. The two cell types of the meta-adenohypophysis (Plate I, fig. 4) are best demonstrated in glands fixed in Elftman's chrome alum fluid. One cell type is a cyanophil but the other cannot be classified on the basis of its staining reaction.

Cyanophilic cells are round or oval with similarly shaped nuclei (length, 5.0 to 7.4 micra; width, 3.0 to 6.0 micra; mean nuclear length, 4.0 micra). The cytoplasmic granules, which are comparatively large, are subject to variation in color with Heidenhain's azan technique, depending on the method of fixation. With formal-sublimate the granules are purple, with chrome alum fluid they assume a very deep blue hue, and with

Helly's solution they are a more reddish-purple. Large aggregates of red-staining chromatin occupy the center of the nucleus. The nucleolus also takes azocarmine stain.

The second cell type of the meta-adenohypophysis exhibits inconsistent staining reactions. It occurs in nests and is generally spindle-shaped, but may show considerable variation in form (length, 5.0 to 8.0 micra; width, 2.0 to 5.5 micra). The nuclei (average length, 4.1 micra) are bizarre, with inconspicuous nucleoli. Generally the cytoplasm appears to be agranular and translucent. The staining reaction of these cells is very erratic, *i.e.,* they are non-selective for any dyes. Pastel hues, shades of green and brown and odd mixtures of red, orange and blue are evident in any small group of them. Their cellular and nuclear membranes also lack definition.

d. Neurohypophysis

The neurohypophysis occupies a sizable portion of the gland and possesses many interesting and distinctive features. The typical impression is one of a highly branched or loosely tangled network of fine fibroid material that is continuous with the infundibular stalk and extends into the glandular epithelium. This is generously interspersed with granular material, large irregularly shaped amorphous masses and large nuclei.

The coarse granular material, usually considered to be neurosecretory, stains red with azocarmine and a muddy, light orange-red with ponceau-acid fuchsin. The heaviest concentration of it is consistently found in the neurohypophysial region adjacent to the meta-adenohypophysis where the granules may be so numerous that the tangled network appearance of the neurohypophysis is obscured (Plate III, fig. 1). The granules are scarce or absent in the region of the pro-adenohypophysis, but become more numerous posteriorly. Neurosecretory granules are concentrated adjacent to, but never in, the vascular channels.

The amorphous masses, or "Herring bodies," are similar to the neurosecretory granules in their staining response and are located in the mid-dorsal region. The smaller ones are uniformly colored red, but in the larger masses a darker peripheral surrounds a lighter central area.

The rounded nuclei, which are characteristic of the neurohypophysis, are relatively large (about six micra). They possess a heavy nuclear membrane which encloses a carminophilic nucleolus and chromatin particles. The cytoplasm is not demonstrable.

A lamina of ependyma cells lines the neurohypophysial area bordering the infundibular cavity and the hypophysial recess. These cells have a large nucleus with a modicum of cytoplasm which extends as finger-like processes into the lumen.

3. Observations with periodic acid-Schiff and aldehyde fuchsin methods.

a. Pro-adenohypophysis

Application of the periodic acid-Schiff (PAS) and the aldehyde fuchsin techniques indicates a general negative response within the pro-adenohypophysis. There is, however, a small PAS-positive sphere, smaller than a nucleolus, just outside the nuclear membrane. These positively-stained granules have been described in the stickleback and referred to as "juxta-nuclear granules" by Van Mullem (1958).

b. Meso-adenohypophysis

All PAS-positive cells of the meso-adenohypophysis are also cyanophilic. The strongest concentration of PAS-positive material is found in the peripheral meso-adenohypophysis with less in the central portion (Plate III, fig. 2). These areas correspond respectively to the peripheral layer of cyanophils and to the cyanophils of the mid-region. Ventral cyanophils contain numerous large, PAS-positive granules, small, clear areas presumably vacuoles, positive colloid droplets and non-staining nuclei. Two cell types can be identified in the mid-region; cyanophils with fine PAS-positive material and acidophils that are negative. As with the pro-adenohypophysial acidophils, a single PAS-positive granule lies adjacent to the nuclear membrane. In the meso-adenohypophysis, as in all other parts of the platyfish hypophysis, blood cells and blood vessels cannot be demonstrated with Schiff's reagent.

In Halmi's (1952) method, light green and aldehyde fuchsin differentiate gonadotrophs from thyrotrophs in the rat and mouse, and orange G demonstrates acidophils. In the platyfish pituitary, however, purple granular substance (aldehyde fuchsin-positive) is found in all cells that are PAS-positive (Plate III, figs. 1 & 2). The colloid droplets in the peripheral cyanophils of the meso-adenohypophysis do not accept aldehyde fuchsin; they do, however, stain with orange G. This affinity for orange G is also characteristic for nucleoli, red blood cells and the acidophils of the meso-adenohypophysis. Cyanophils of the dorsal meso-adenohypophysis may contain clumped purple granules in a homogeneous green cytoplasm.

Elftman (1959) reports that aldehyde fuchsin following oxidation demonstrates both thyrotrophs and gonadotrophs in mammalian pituitaries and that without oxidation, only the cells concerned with synthesis of thyrotrophin are purple. In the pituitaries of sexually mature platyfish, all cyanophils stain purple with or without prior oxidation. The oxidative process merely increases the intensity of the aldehyde fuchsin response.

c. Meta-adenohypophysis

The cyanophils of the meta-adenohypophysis are colored light red with the PAS technique and purple with Halmi's aldehyde fuchsin. As with the azan method, agranular cells of the meta-adenohypophysis show no consistent staining reaction with PAS and aldehyde fuchsin procedures.

d. Neurohypophysis

The neurosecretory granules and Herring bodies do not respond to the periodic acid-Schiff method but are dramatically demonstrated with aldehyde fuchsin (Plate III, figs. 1 & 2). Few aldehyde fuchsin-positive granules may be identified in the ependyma cells lining the infundibular cavity.

4. Differential Cell Counts

Differential cell counts of sexually mature male and female platyfish do not show any sexual dimorphism *i.e.*, male and female counts were not significantly different. The mean percentages for both sexes were: acidophils, 50.0%; cyanophils, 20.0%; chromophobes, 30.0% (see Table VI).

B. Pituitary Structure in Relation to Age

1. The Hypophysis in Young Forms (One to Eight Weeks Old).

In contrast to the hypophysis of mature fish, the glands of one-week-postpartum specimens are ellipsoidal in shape and lack the dorsal depression and ventral convexity. The immature gland is held against the brain by a very wide, thin-walled stalk and lies just dorsal to the parasphenoid bone (Plate II, fig. 1).

In mid-sagittal section, the pituitary gland is composed of almost equal areas of adenohypophysis and neurohypophysis. The adenohypophysis exhibits two clearly separated areas: an anterior portion, occupying about two-thirds of the gland's total length, and a posterior third, the meta-adenohypophysis. The anterior portion, in turn, may be further sub-divided by cell type into an anterior two-thirds composed of carminophilic acidophils representative of the pro-adenohypophysis, and a posterior third populated by groups of mixed acidophils and cyanophils and comparable to the adult meso-adenohypophysis. In one-week-old fish, the wedge of chromophobic cells and the connective tissue septum, which separate the pro- and meso-adenohypophysis in adults, are dorsal to the pro-adenohypophysis and form the boundary between this region and the neurohypophysis. Moreover, the wedge of cells is quite small and the connective tissue septum appears to end blindly in the mid-dorsal region.

The more extensive anterior acidophilic area is composed of large cells with coarse carminophilic granules and a large round or oval nucleus with a prominent nucleolus. Dispersed among these acidophils are some chromophobes that stain light blue or are colorless.

Posterior to these anterior acidophils is the region that encompasses cyanophils and acidophils in varying proportions and is comparable to the adult meso-adenohypophysis. The cyanophils are large and circular with unevenly distributed fine cytoplasmic granules. These cells are structurally similar to, and probably identical with, the central cyanophils of the mature platyfish. The acidophils are pyramidal or spherical in shape and contain large, red-orange granules. The peripheral cyanophils (ventral in mid-sagittal section), characteristic of the adult pituitary, are not present at this stage of development. Instead, this area contains a few chromophobes (Plate II, fig. 3).

The meta-adenohypophysis, directly ventral to the neurohypophysis, is a dense aggregate of typical purple cyanophils and limited numbers of spindle-shaped cells with little affinity for azocarmine.

The neurohypophysis of both week-old fish and adult is essentially similar, but it occupies most of the dorsal mass of the immature pituitary and lacks the extensive arborization found in the adenohypophysis of the adult. In the region of the hypophysial recess of week-old fish, the cells exhibit a thin regular rim of cyanophilic cytoplasm. The large nuclei, characteristic of the adult neurohypophysis, are absent.

The developmental picture that is presented by the pituitary glands of males and females up to four weeks of age remains essentially unchanged, although there is a perceptible increase in hypophysial size with the increase in body length (see Table IV). Acidophils are still the dominant cell type of the gland. A small band of chromophobes, about one to two cells in depth along the ventral periphery of the meso-adenohypophysis, represents the corresponding cyanophilic region in the adult gland. With increase in age, the nuclei of these chromophobes show a progressional increase in volume. During the initial two- to four-week period of development, the meta-adenohypophysis proliferates posteriorly and dorsally from its original ventral position. Neurohypophysial growth is most obvious in the region of the meso-adeno-

TABLE III. GLAND REGION MEASUREMENTS (IN MICRA)

			Mature Female	Mature Male	Mature Male & Female	Castrate Male	Gravid Female	Thyroid Tumor	Tumor Control	Melanoma	Melanoma Control
PRO.*	Length	Mean	223.4	198.0	214.9	213.1	207.3	199.3	171.5	212.1	181.5
		S.E.	14.0	16.0	10.7	6.5	16.8	8.1	15.7	25.0	8.4
		No. Animals	10	5	15	7	4	6	6	7	4
	Width	Mean	172.1	155.0	166.4	213.0	242.5	225.8	166.7	250.1	204.0
		S.E.	9.0	16.0	10.7	17.4	28.0	13.2	11.8	24.0	10.4
		No. Animals	10	5	15	7	4	6	6	7	4
MESO.	Length	Mean	188.1	171.8	180.7	293.7	233.0	268.7	166.2	178.7	153.7
		S.E.	27.8	15.5	14.2	19.2	24.1	10.9	22.7	18.7	8.8
		No. Animals	6	5	11	7	4	6	6	7	3
	Width	Mean	115.1	88.3	103.7	181.3	146.5	160.5	101.7	164.7	120.7
		S.E.	12.8	14.9	10.2	12.5	21.3	15.1	6.2	16.6	5.9
		No. Animals	6	3	9	7	4	6	6	7	3
META.	Length	Mean	109.8	126.2	116.4	159.8	155.3	122.8	148.7	102.7	147.3
		S.E.	8.1	8.0	7.0	24.7	26.9	20.1	14.8	7.6	15.4
		No. Animals	6	4	10	5	4	6	6	7	4
	Width	Mean	138.7	124.7	134.4	137.6	154.3	135.3	114.5	126.0	143.0
		S.E.	14.1	27.0	11.6	13.8	29.5	14.1	5.5	11.0	14.9
		No. Animals	9	4	13	5	4	6	6	7	4

*Abbreviations used in the following tables:

PRO.	Pro-adenohypophysis
MESO.	Meso-adenohypophysis
META.	Meta-adenohypophysis
S.E.	Standard error
NO.	Number
ACIDO.	Acidophil
CYANO.	Cyanophil
'PHOBE.	Chromophobe
NUC.	Nucleus
%	Percent.

Table IV. Pituitary Dimensions and Body Length of Young Fish
(Gland Values in Micra)

		Age (in Weeks)					
		1	2	3	4	6	8
Average Total Body Length (mm.*)		10.0	12.0	15.0	17.0	19.5	22.3
	No. Animals	4	4	4	4	4	4
Total	Length	200	257	255	299	359	
	S.E.	13.4	4.6	10.3	4.5	7.9	
	No. Animals	3	3	8	4	3	
Gland	Width	52	73	80	95	103	
	S.E.	2.9	1.7	4.1	7.0	4.2	
	No. Animals	3	3	7	4	3	
	Depth	136	163	182	206	232	
	S.E.	11.8	6.3	11.5	9.8	4.2	
	No. Animals	3	3	7	4	3	
Pro.	Length				158	204	
	S.E.				13.3	21.8	
	No. Animals				2	3	
	Length+meso.	147	193	209	233	284	
	S.E.	9.6	2.6	12.4	8.2	15.5	
	No. Animals	3	3	7	4	3	
	Width	42	54	66	73	89	
	S.E.	2.6	4.6	4.1	9.5	5.0	
	No. Animals	3	3	7	4	3	
Meso.	Length				80	109	
	S.E.				10.9	14.7	
	No. Animals				3	3	
	Width	25	38	43	49	57	
	S.E.	1.7	1.3	1.2	4.5	3.8	
	No. Animals	3	3	7	4	3	
Meta.	Length	45	75	63	70	74	
	S.E.	8.4	2.1	3.3	2.0	5.0	
	No. Animals	3	3	7	4	3	
	Width	11	26	22	39	59	
	S.E.	1.7	2.1	3.6	4.8	0.9	
	No. Animals	3	3	6	4	3	

*Snout to tip of caudal fin.

hypophysis. Septa of neurohypophysial tissue proliferate into the gland and divide this region into characteristic meso-lobules.

At six weeks of age, there are definite indications of a separation between the pro- and meso-adenohypophysis. Although the chromophobic wedge of cells and the connective tissue septum are still essentially dorsally placed, the beginning of their ventral proliferation can be seen between the pro-adenohypophysis and meso-adenohypophysis. This progression is more marked at eight weeks of age when the meso- and pro-adenohypophysis are more clearly separated. The meso-adenohypophysis shows extensive growth along all axes. To a lesser extent, the pro-adenohypophysis has extended anteriorly and dorsally. As a result of this increase in glandular tissue, the neurohypophysis becomes relatively smaller and occupies a more central position.

Cytologically, the most interesting change involves the cells of the peripheral meso-adenohypophysis. In six-week-old platyfish, the first appearance of a few granulated cyanophils occurs in the still predominately chromophobic zone. These cells continue to be characterized by large nuclei and little cytoplasm. In mid-sagittal sections of eight-week-old fish, the ventral zone of the meso-adenohypophysis has noticeably increased in mass (Plate II, figs. 2 & 4). Cyanophilic cells with denser granulation are much more in evidence, and these are small facsimiles of cells from corresponding areas of the mature gland.

At six weeks postpartum, the cyanophils of the central meso-adenohypophysis are more fully granulated and resemble the corresponding cells in adults. Carminophilic acidophils of the pro-adenohypophysis are large and fully granulated; they contrast with the less heavily granulated acidophils of the meso-adenohypophysis. Both cells, however, contain large nuclei with prominent nucleoli. In six- to eight-week-old fish, the meta-adenohypophysis shows a progressive degranulation of the purple cyanophils.

Between the first and sixth week of development, all glandular measurements have approximately doubled (Table IV). During this period there are no significant changes in acidophil and cyanophil ratios (see Table VI). Chromophobes, however, show a slight but significant increase (P equals less than 0.05). On the other hand, the percentage of acidophils in the pituitaries of one- to six-week-old fish remains fairly constant, but higher than the acidophil percentages of mature glands. No mitotic figures were ever recognized during this development.

2. Pituitary Glands of Aging Female Platyfish (28 to 33 Months Old).

The animals utilized for studies of the effects of aging on the pituitary gland were aged and control platyfish of the Grijalva strain selected from several laboratory aquaria.

The aged pituitary is suspended by a thick-walled, wide stalk and is surrounded by a thick connective tissue capsule. In mid-sagittal section the gland appears rounded because of an obvious increase in the dorsal-ventral axis (width) of each region of the gland. With this increase in width, the pro- and meta-adenohypophysis seem to have become compressed along their anterior-posterior axis (Plate IV, fig. 1). The pro- and meso-adenohypophysis and neurohypophysis are the regions that exhibit the most noticeable changes from the normal.

The pro-adenohypophysis of aged females is comprised of a large ventral chromophobic area not found in normal younger fish, and a carminophilic area dorsal to it. Cell counts in senile fish (Table VI) reflect this chromophobia. Nuclei of the chromophobes are irregular in shape and have a relatively thick membrane. Nucleoli show considerable variation. As indicated in the table of cell dimensions, carminophils are larger (Table V) and they are richly granulated with vivid, dark carmine inclusions.

Characteristically, the meso-adenohypophysis has a wider peripheral band of cyanophils. This increase in area represents cellular hypertrophy (Table V, P=less than 0.05) rather than a cell increment. The cytoplasm of these larger cyanophils is usually pale blue and hyaline. In cells with granular inclusions, the extent of granulation is highly variable. Some vacuoles and an abundance of red- or orange-staining bodies are contained in the cyanophils of the peripheral meso-adenohypophysis. Nuclear size varies and the nuclei contain fine cyanophilic chromatin that surrounds a conspicuous nucleolus. The cyanophils of the central meso-adenohypophysis significantly decrease in size (P=0.05, see Table V) as also do their nuclei. Cytoplasmic appearance varies considerably but generally the cells display a loss of granular material. Vacuolation is infrequent. The acidophils of the meso-adenohypophysis of old glands show no apparent morphological changes.

As revealed in sagittal sections, the neurohypophysis of glands from senile fish occupies a much greater area and its embranchment is more marked. There is a definite increase in the size of the Herring bodies.

C. Effects of Gravidity on the Pituitary of Platyfish

Fish were taken from laboratory stock and if found gravid on routine examination, were utilized for this phase of the investigation. No record of the advancement of gestation was known at the time of sacrifice. Nevertheless, in the seven gravid platyfish examined, certain consistencies prevailed in the histology and cytology of their pituitary glands.

The meso-adenohypophysis of gravid fish is wider and longer than it is in normal females (see Table III). This increase in mass is brought about by hypertrophy and hyperplasia of the peripheral cyanophils and hyperplasia, without hypertrophy, of the acidophils in the central region (Plate V, figs. 1 & 2; Table V). The cyanophil count increases from 20.0% in normal females to 29.0% in gravid individuals. Often, the cyanophils of the peripheral region will extend as a thin band ventrally and posteriorly around the meta-adenohypophysis. The cyanophils of the central zone exhibit no gross variations from those in non-gravid females. Peripheral cyanophils contain many large, spherical, reddish colloid droplets, and clear vacuoles in an agranular-appearing cytoplasm. Round nuclei enclose large red nucleoli. Acidophils are the predominant cell of the dorsal meso-adenohypophysis. They are round or oval in form and have similarly shaped nucleoli. Their coarse, red-orange granulation makes them readily indentifiable.

Other parts of the pituitary gland are not noticeably altered in gravid platyfish.

D. Effects of Castration on the Pituitary of Male Platyfish

In castrated male platyfish, the hypophysis is always characterized by a marked increase in total size (mean dimensions: length, 567.7 micra; width, 249.5 micra; depth, 511.8 micra). Although the size of the pro- and meta-adenohypophysis is essentially unchanged, the meso-adenohypophysis increases markedly along all its axes (Table III). Very often the meso-adenohypophysis encroaches upon the ventral and posterior meta-adenohypophysis with a thin border of ventral meso-adenohypophysial cyanophils.

The cyanophil is the predominate type of cell in the meso-adenohypophysis of castrate males. Differential cell counts corroborate this increase in cyanophils (see Table VI). The castrate pituitary invariably exhibits an increase in the width of the peripheral cyanophil cell layer (Plate III, fig. 3). Although all types of cyanophils are affected by gonadectomy, only the cyanophils of the peripheral layer are conspicuously vacuolated. In the central meso-adenohypophysis, the small islets of cells, formed by the septa of neurohypophysial tissue in the normal gland, are less obvious; instead, large masses of cells constitute this region. Acidophils are oriented in small groups along the neurohypophysial border and as isolated cells scattered among the central cyanophils. In addition, there appears to be an increased amount of vascularity.

The peripheral cyanophils of the meso-adenohypophysis exhibit pronounced hypertrophy and hyperplasia (Table V). It is therefore surprising that no mitotic figures were ever observed. The majority of the peripheral cyanophils show varying degrees of cytoplasmic degranulation and inflated nuclei and nucleoli. Vacuoles vary in magnitude and number and are evident in the majority of these cyanophils. They generally stain a paler blue than corresponding cells of normal fish, and cell delineation is less pronounced. In many castrate pituitaries, cells that fit the description of mammalian "castration cells" were observed. These cells are not present in large numbers but are readily identifiable. Their most prominent characteristics are the large, pale blue, agranular vacuole, the thin ring of cytoplasm containing dark granules, and the nucleus displaced to the periphery, all of which give the cell the signet-ring appearance reminiscent of its mammalian counterpart (Plate III, fig. 4).

The mid-dorsal cyanophils are irregularly shaped (normally these cells are smooth spheres) and only slightly hypertrophied (Table V). The granules appear as dispersed light blue floccules. Vacuolation is usually absent. Nucleoli are more prominent in these cells than in their counterparts in unoperated fish.

Periodic acid-Schiff and aldehyde fuchsin techniques confirm the observations made on azan stained castrate pituitaries. The amount of "positive" material was reduced, corresponding to the degranulation that occurs in both cyanophil cell types of the meso-adenohypophysis. This degranulation was especially obvious in the cells of the peripheral zone. In contrast, the response of the cyanophils in the meta-adenohypophysis to the aldehyde fuchsin and the PAS techniques remains unchanged.

The acidophils of the meso-adenohypophysis are essentially the same as those in the gland of intact fish.

In the pro- and meta-adenohypophysis, and neurohypophysis, there are no significant deviations from the unoperated controls.

E. The Pituitary in Tumor-bearing Fish

1. Platyfish with Heterotopic Thyroid Tumors

Pituitaries from platyfish with heterotopic thyroid tumors, in addition to being larger than normal, can be readily identified macroscopically by a characteristic bulging of the meso-adenohypophysis that girdles the mid-region of the gland (Text-fig. 1B). Microscopically it can be seen that the marked increase in the dimensions of the meso-adenohypophysis accounts for the increased total volume of the pituitary (Tables II & III; Plate IV, fig. 2). Peripheral and central cyanophils are clearly delineated. Peripheral cyanophils are confined to the protuberant region where they occur as loose aggregates of dark blue cells (Plate IV, fig. 3). Cells of the central meso-adenohypophysis, located between the peripheral cyanophils and the neurohypophysis, comprise two types: closely packed, rounded cyanophils that are the predominant type, and orange acidophils that occur singly or in small groups along the neurohypophysis. These clusters of dorsal meso-adenohypophysial cells are much larger than those in the normal adult.

The cyanophils of the central region show hypertrophy and hyperplasia. Measurements indicate that both nuclei and cell size are involved in the hypertrophy (Table V). No mitotic fig-

TABLE V. CELLULAR DIMENSIONS AND NUCLEAR LENGTH

(Cell dimension values represent the mean products of cell length × cell width in micra. Mean nuclear length given in micra)

	Mature Female	Mature Male	Mature Male & Female	Castrate Male	Gravid Female	Thyroid Tumor	Tumor Control	Melanoma	Melanoma Control	Old Females	Old Controls
PRO.											
ACIDO.											
Mean	35.1	35.8	35.3	38.6	35.0	38.5	35.6	49.2	37.6	45.4	33.2
S.E.	2.0	1.7	1.3	1.5	4.0	4.3	3.3	2.8	2.0	2.9	1.2
Nuc.	4.2	4.7	4.4	4.6	4.8	4.8	4.4	4.9	4.2	4.6	4.5
S.E.	0.1	0.2	0.1	0.1	0.2	0.1	0.3	0.3	0.2	0.1	0.2
No. Animals	10	5	15	7	3	6	6	6	4	6	5
'PHOBE.											
Mean	18.9	20.4	19.5								
S.E.	2.4	3.9	2.0								
Nuc.	3.4	3.5	3.5								
S.E.	0.2	0.3	0.1								
No. Animals	8	5	13								
MESO.											
ACIDO.											
Mean	32.8	26.4	31.2	27.4	30.0	35.0	28.8	46.0	38.5	28.6	28.8
S.E.	2.8	7.2	2.4	0.5	3.4	1.6	3.7	3.1	6.0	3.2	3.8
Nuc.	4.2	3.7	4.1	3.8	4.6	4.3	3.9	4.7	4.3	3.6	4.1
S.E.	0.1	0.1	0.1	0.1	0.3	0.2	0.1	0.1	0.4	0.1	0.7
No. Animals	9	3	12	7	4	6	6	7	4	5	4
*CYANO.											
Mean	24.4	23.4	24.0	33.3	22.3	62.4	22.9			21.1	28.0
S.E.	1.0	3.4	1.1	1.9	1.3	4.1	1.9			2.9	0.8
Nuc.	3.9	3.8	3.8	4.2	4.0	5.4	3.8			3.4	4.6
S.E.	0.1	0.1	0.1	0.2	0.1	0.2	0.3			0.3	0.1
No. Animals	10	5	15	7	4	6	6			5	5

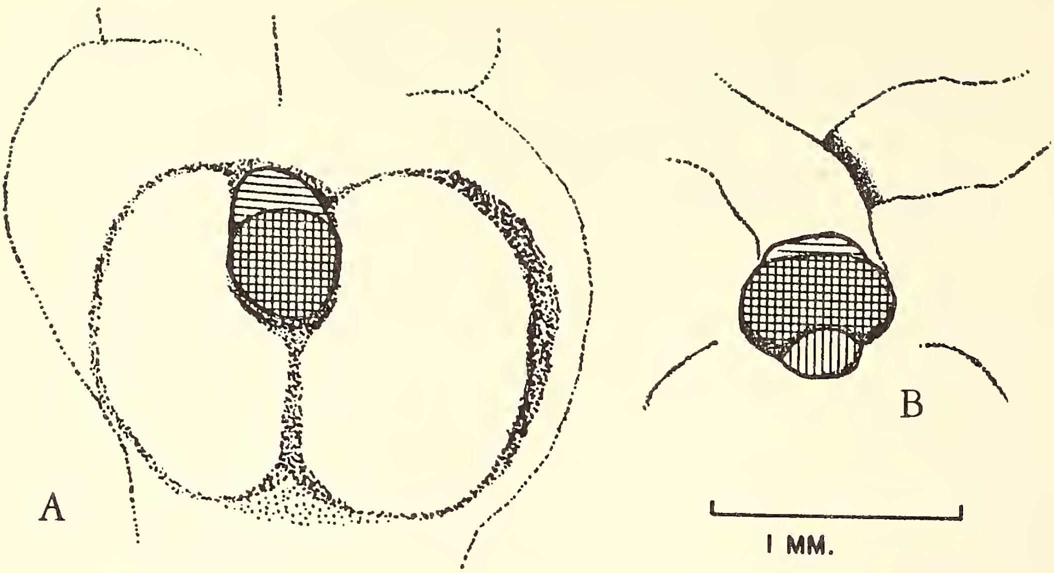
*Central cyanophils.

TABLE V. CELLULAR DIMENSIONS AND NUCLEAR LENGTH (Contd)

(Cell dimension values represent the mean products of cell length × cell width in micra. Mean nuclear length given in micra)

	Mature Female	Mature Male	Mature Male & Female	Castrate Male	Gravid Female	Thyroid Tumor	Tumor Control	Melanoma	Melanoma Control	Old Females	Old Controls
MESO.											
†CYANO											
Mean	39.6	32.7	37.2	54.6	52.8	26.6	35.0			50.7	36.6
S.E.	3.9	2.9	2.2	3.3	2.4	1.3	1.9			6.0	2.9
Nuc.	4.3	4.1	4.2	4.5	4.3	3.8	4.5			4.2	4.0
S.E.	0.1	0.1	0.1	0.2	0.3	0.1	0.1			0.3	0.3
No. Animals	10	5	15	7	4	6	6			6	5
'PHOBE											
Mean	21.8	19.4	21.1								
S.E.	2.4	4.6	2.0								
Nuc.	3.9	4.0	3.9								
S.E.	0.3	0.4	0.2								
No. Animals	7	3	10								
META.											
CYANO.											
Mean	29.1	31.2	29.8					34.9	29.0	25.6	27.1
S.E.	3.1	2.9	1.9					2.1	2.0	3.1	3.7
Nuc.	4.1	3.8	4.0					4.7	4.3	3.6	2.9
S.E.	0.2	0.2	0.1					0.1	0.4	0.1	0.1
No. Animals	10	5	15					6	4	4	3
"TYPE II"											
Mean	25.3	22.4	24.1								
S.E.	2.6	3.1	1.5								
Nuc.	4.3	3.8	4.1								
S.E.	0.3	0.2	0.1								
No. Animals	7	5	12								

†Peripheral Cyanophils.



Text-fig. 1. **A.** Normal pituitary gland in ventral aspect with associated brain structures (stippled lines). Pro-adenohypophysis (anterior) represented by horizontal lines; squares indicate combined meso- and meta-adenohypophysis. Camera lucida drawing. **B.** Pituitary gland of a playfish with a thyroid tumor. Note bulging of meso-adenohypophysis (squares). Horizontal lines represent the pro-adenohypophysis (just posterior to the optic chiasma); vertical lines represent the meta-adenohypophysis. Camera lucida, ventral aspect.

ures were ever observed. Red nucleoli are also larger and more prominent. There is a very marked dimunition of cytoplasmic granules, and this is confirmed by a diminished aldehyde fuchsin reaction (Plate IV, figs. 3 & 4). Small vacuoles appear infrequently. Acidophils of the central meso-adenohypophysis of tumorous fish are larger; however, statistical analysis indicates that this is not a significant difference. These acidophils are also less regular in shape.

Peripheral cyanophils of the meso-adenohypophysis are smaller (P=less than 0.01), more irregularly shaped, and more intensely cyanophilic and aldehyde fuchsin-positive. The intensely staining, heavily granulated cytoplasm contains varying numbers of red or orange, aldehyde fuchsin-negative droplets, as in the normal fish. Quite characteristic are the nuclei; they are smaller than normal, less uniform in shape, more condensed, and have smaller nucleoli.

In one specimen, a fish in which the neoplasm appeared to be confined to the gill region, the protuberant area of the meso-adenohypophysis was less obvious and hypertrophy of the central cyanophils was evident without distinct hyperplasia.

The other three regions of the pituitary show no marked histological modifications from the picture presented by glands of the normal fish.

2. Hybrids with Melanotic Tumors

The most prevalent and consistent features of the melanomatous fish hypophysis are a highly significant (P=0.01) hypertrophy of the pro-adenohypophysial acidophils and a dramatic hyperplasia of the meso-adenohypophysial acidophils.

The pro-adenohypophysial carminophils in fish with melanoma may assume one or the other of two cytological states. In some glands, all the acidophils are very much degranulated except for those cells that are adjacent to the blood vessels. In other glands, all the acidophils are distended with numerous closely packed coarse granules. Both forms of carminophils have a large nucleus and a prominent nucleolus.

So marked is the acidophil hyperplasia in the meso-adenohypophysis that at low magnification it appears as a wide, bright, orange-yellow band extending between the pro- and meta-adenohypophysis (Plate V, fig. 4). These cells are almost always fully granulated. The majority have a homogeneous-appearing granulation; occasionally coalescence of the granules seems evident. Cell and nuclear shapes are polymorphic. The prominent nucleolus is variable in size. Although the acidophils of the mid-region are larger on the average than those of normal animals, the large range of values precludes any

TABLE VI. DIFFERENTIAL CELL COUNTS (WHOLE GLANDS)

	ACIDO.			CYANO.			'PHOBE.			Mean No. Counted Per Gland
	Mean %	S.E.	No. Animals	Mean %	S.E.	No. Animals	Mean %	S.E.	No. Animals	
Mature Males & Females	50.0	0.7	9	20.0	0.7	9	30.0	0.9	9	1190
Mature Males	49.3	0.9	3	20.0	2.1	3	30.7	1.3	3	1199
Mature Females	50.3	1.1	6	20.0	1.1	6	29.7	1.4	6	1186
Castrate Male	40.6	0.6	7	32.7	0.8	7	26.7	0.9	7	1436
Thyroid Tumor	44.6	0.9	5	30.0	0.7	5	25.4	0.5	5	1287
Tumor Control	47.9	1.2	7	21.1	0.6	7	31.0	1.1	7	1223
Gravid Female	48.3	1.1	4	29.0	2.3	4	22.7	2.0	4	1692
Old Female	33.8	3.9	4	26.8	4.5	4	39.4	4.8	4	941
Old Control	48.3	0.9	4	25.3	0.9	4	26.4	0.9	4	718
IMMATURE										
One Week Old	56.7	1.7	3	20.3	1.3	3	23.0	1.3	3	355
Two " "	55.7	0.4	3	19.0	0.0	3	25.3	0.4	3	566
Three " "	54.3	1.2	7	20.0	1.1	7	25.7	0.9	7	545
Four " "	53.0	2.1	3	17.4	0.4	3	29.6	2.6	3	824
Six " "	53.7	1.3	3	19.0	0.9	3	27.3	0.4	3	1130

statistical significance (*viz.*, 46.0±3.0 versus 38.5± 6.0).

Less consistent, but nevertheless quite apparent, is the increased size of meta-adenohypophysial cyanophils. Here, too, the cytoplasm is completely granulated. These cells average larger in size than the corresponding cells of normal animals, but determination of "P" values (greater than 0.05) indicates that there is no statistically significant increase. The agranular cells of the meta-adenohypophysis are round, stain light green with the azan method, and possess dense nuclei.

The variability in gland measurements (Table II) is great, but this may be explained, at least in part, by the animals used. In "M1" animals, which were produced by repeated back-crosses to the swordtail, the pituitaries resemble in gross shape the swordtail pituitary, that is, they have distinctly rounded (knob-like) anterior and posterior ends. In the "M2" strain, in which hybrid offspring were inbred, the posterior end is more tapered and thus resembles the platyfish pituitary. The only statistically significant differences in pituitary dimensions between melanomatous and normal fish are a decrease in length of the meta-adenohypophysis and an increase in the dorsal-ventral axis (width axis) of the pro- and meso-adenohypophysis (Table III) in fish with melanomas.

Discussion

A. The Pituitary in Normal, Sexually Mature Platyfish

Most investigators agree that the teleost pituitary may be differentiated into four regions—three glandular portions and a neurohypophysis—but views differ concerning the precise location of the regions, their characteristic cell types and terminology. The use of diverse histological procedures, particularly fixatives and stains designed for general use, has contributed to this confusion, because the glandular regions are usually delineated by the cells they are supposed to contain. For example, Mathews (1936) found only two glandular areas in pituitaries of *Fundulus heteroclitus*. His failure to differentiate a pro- and meso-adenohypophysis in his "transitional area" very likely resulted from his choice of eosin-azure as a stain. With aldehyde fuchsin (Halmi's method), Sokol (1961) demonstrated three glandular regions in the same species. Her descripition lends support to the present interpretation of the arrangement of hypophysial regions in *X. maculatus*.

The importance of technique is also illustrated by Bell's (1938) description of the goldfish hypophysis. He stated that the Masson-stained "pars anterior" (pro-adenohypophysis) was largely "basophilic." This is very questionable in the light of Levenstein's (1939) and Scruggs' (1939) reports in which the Heidenhain azan technique was used, and in a more recent report in which erythrosin and anilin blue were utilized (Olivereau, 1962a). All three investigators agree that the pro-adenohypophysis (our terminology) is predominately acidophilic with some basophils (cyanophils) and chromophobes. Ortman (1961) identified "dull purple basophils" in Mallory-stained pro-adenohypophyses of the closely related carp. In the present investigation, no cyanophils have been demonstrated in the carminophilic pro-adenohypophysis of *X. maculatus*.

In an investigation of Helly-fixed and Masson-stained pituitary glands of six species of poeciliids (not including *X. maculatus*), Potts (1942) reported that the pars intermedia (meta-adenohypophysis) surrounds the meso-adenohypophysis, and is contiguous with the pro-adenohypophysis. In normal platyfish, the meta-adenohypophysis never surrounds the meso-adenohypophysis, and the reverse may be true in pituitaries of castrate males and gravid females. The differentiation of the meta-adenohypophysis from the peripheral meso-adenohypophysis can be accomplished only by the identification of the characteristic cyanophils of each region. It appears that Potts did not achieve this separation of cell types; he stated that the "basophils" of the middle pars intermedia (meta-adenohypophysis) closely resemble the basophils of the "Übergangsteil" (meso-adenohypophysis).

Öztan (1961) studied platyfish pituitaries fixed with Bouin's solution containing trichloracetic acid and subjected to several suitable staining techniques. Neither the meso- nor the meta-adenohypophysis appears to have been properly differentiated, however, and as a result they were combined into a "central zone of the pars distalis." As a result of this difficulty, the pars intermedia (meta-adenohypophysis) seems to have been assigned a questionable location in the gland. According to our interpretation, Öztan's "pars intermedia" partly corresponds to the chromophobic wedge of cells adjacent to the pro-adenohypophysis; indeed, Öztan reports that these cells are chromophobic in their staining affinities. We must now account for the several cyanophil cell types described by this investigator. The position and description of her "type 2" cells correspond closely to the central meso-adenohypophysial cyanophils that we have described. On the basis of position, her "type 1" cells belong to the meta-adenohypophysis. An examination of Öztan's chart for tinctorial properties and her description of "type 1" cells reveal the similarities between these cells and the second cell type of the meta-adenohypophysis that we have described. Öztan's "type 3" basophilic cell resembles, for the most part, our peripheral cyanophils of the meso-adenohypophysis, but she does not distinguish them from the cyanophils of the meta-adenohypophysis.

Baker-Cohen (1961) and Mac Intyre & Baker-Cohen (1961) have provided brief descriptions of the platyfish pituitary in reports primarily devoted to other aspects of fish endocrinology. Aside from their brevity, these studies illustrate the impracticability of utilizing general histological techniques for specialized pituitary analyses. Their information was derived from sections 10 micra thick of whole fish fixed in Bouin's solution or 10% formalin, decalcified in formic acid and stained with hematoxylin and eosin or with the Masson method. Only a "dark basophil" in the most anterior region, a "paler basophil" in the mid-region, and the neurohypophysis could be differentiated. These authors could not identify acidophils. It appears that the "dark basophils" of the "anterior pituitary" are actually carminophilic acidophils (with Heidenhain's) of the pro-adenohypophysis.

Exception must be taken to the statement of McManus & Mowry (1960, p. 318) that "fixation for the anterior pituitary is of some importance when enzyme activity is to be studied but it does not seem crucial in the differentiation of cell types." This statement was based on the examination of human material and is very likely only applicable to human glands. The work of Geske (1956) may be cited as evidence that the view of McManus & Mowry does not necessarily hold for the teleosts. In azan-stained, Susa-fixed pituitary glands of the guppy, *Poecilia (=Lebistes) reticulata,* Geske described a meso-adenohypophysial "chromophobic zone" that fluctuated in size and cell number in reponse to sex hormone administration. His description indicates that this area corresponds to the ventral cyanophilic zone (meso-adenohypophysis) of the platyfish. It is conceivable that one might infer the presence of such a chromophobic zone even in the platyfish but only in Susa-fixed, azan-stained specimens. When it was ascertained that this response was not obtained with other methods of fixation (*e.g.,* formol-sublimate, chrome alum, etc.), we decided to eliminate the use of the Susa fixative as well as others containing acetic acid (see p. 219). The cyanophils of the ventral zone of the meso-adenohypophysis appear to be especially vulnerable to improper fixation. Vervoort (1957), also employing Susa-fixed, azan-stained guppy pituitaries, could not demonstrate acidophils in a region that corresponds to the acidophil-containing mid-dorsal region of the meso-adenohypophysis in *X. maculatus.* Another example of the interdependence of fixation and staining is the reaction of meta-adenohypophysial cyanophils to anilin blue following various fixatives (see p. 221).

Dempsey & Wislocki (1945) and Purves & Griesbach (1957), as well as others, have pointed out the fallacy of using the term "basophil" with trichrome staining because all these stains are acid stains and true basophilia cannot be indicated with their use. Vervoort (1957) went so far as to assert that it may be impossible to characterize any cells by specific staining reaction since this is dependent on such variables as previous fixation, pH of the stains, and duration of staining. In the present investigation, the term *acidophil* is applied to those cells that possess granules exhibiting an affinity for azocarmine and/or orange G and are PAS- and aldehyde fuchsin-negative. *Cyanophils* are cells containing cytoplasmic granules that stain with anilin blue and are positive for the PAS and aldehyde fuchsin reactions. *Chromophobes* are refractory or faintly staining cells.

There is one cell type, the agranular, generally spindle-shaped cell of the meta-adenohypophysis, that evades classification by the above tinctorial standards. From observations on its staining response (see p. 222), one cannot help but draw the analogy of the cell cytoplasm to a sponge that will take up any dye in solution presented to it. It is not likely that this represents an artifact, for this type of reaction was manifested with all of the fixatives and staining procedures used. This cell type may represent a degenerate or intermediate cell from the meta-adenohypophysis—and perhaps the meso-adenohypophysis as well. In various species of teleosts "acidophils" have been identified in the meta-adenohypophysis (Scruggs, 1939; Kerr, 1942, 1948), but no such cellular elements have been recognized in the meta-adenohypophysis of the platyfish.

B. The Pituitary Gland in Platyfish One to Eight Weeks Old

The thyroid gland of the embryonic guppy undergoes gradual increase in activity during the terminal stages of gestation (Stolk, 1951). A similar state of affairs is also indicated by the report of Tavolga (1949) in which he stated that the thyroid is developed and functional in the embryonic platyfish. Tavolga found no evidence of sexual dimorphism in the gonads nor any indication of somatic cells transforming into germ cells up to the time of birth. In the pituitary gland of one-week-old platyfish, only the dorsal cyanophils of the meso-adenohypophysis are in evidence; no peripheral cyanophils are present. It is generally accepted that a cyanophilic cell type is responsible for the elaboration of TSH. Presumably, the cyanophils of one-week-old platyfish, which are structurally similar to the cyanophils of the adult dorsal meso-adenohypophysis, are related to the activity of the thyroid gland.

On the other hand, the cyanophils of the peripheral meso-adenohypophysis first appear in six-week-old fish as a few sparsely granulated cells dispersed among more numerous chromophobes. At eight weeks postpartum, a thin ventral layer (in sagittal section) composed of fully granulated cyanophils has developed. At six weeks postpartum, there is also a thickening and elongation of the anal fin in males, the beginning of the gonopodium. Experiments involving castration and sex hormone administration have demonstrated that this structure is formed under the influence of androgens (Pickford & Atz, 1957, pp. 86, 180). Coincident with the increase in number of fully granulated ventral meso-adenohypophysial cyanophils, at eight weeks postpartum male and female platyfish show signs of sexual maturity, that is, sperm and oocytes are present in the gonads. It is felt, therefore, that the cyanophils of the ventral meso-adenohypophysis, which are also strikingly affected by castration, are concerned with gonadal maturation and sexual maturity and thus involved in the production of gonadotrophin. The time of appearance of the two classes of cyanophils in the platyfish meso-adenohypophysis does not differ markedly from that indicated for the guppy. In the latter species, Sokol (1956) found the cyanophils of the ventral region appear several weeks later than those of the central meso-adenohypophysis.

Intermedin, which is implicated in the production of melanophores, is stored, if not elaborated, in the meta-adenohypophysis (Pickford & Atz, 1957, p. 44). The meta-adenohypophysis is a comparatively large glandular region in one-week-old platyfish. This observation may be related to the fact that pigmentation occurs early in the embryogenesis of this species (Tavolga, 1949). The expression of the spotted dorsal gene *(Sd)*, evident at birth as light pigmentation on the dorsal fin, becomes quite marked at six weeks of age. At this time, a noticeable degranulation of the purple cyanophils in the meta-adenohypophysis is evident. These observations suggest that these two events may be related.

There still remains the question of the significance of the large population of acidophils characteristic of pituitary glands in young platyfish. From the evidence to be presented, it does not seem unreasonable to assume that at least part of their function is involved in growth. During the first eight weeks of postpartum development, the average total length of the fish more than doubles. Present evidence indicates that a class of acidophils is concerned with the production of growth hormone (Barrnett, Siperstein & Josimovich, 1956), and there is no doubt that a growth hormone is present in pituitary glands of teleosts (Pickford & Atz, 1957, p. 91). In salmon parr and smolt, for example, Olivereau (1954) has demonstrated that there is an intense fuchsinophilia with Masson trichrome preceding periods of marked growth.

C. Aging Effects on the Pituitary Gland of Platyfish

The major problem in conducting aging studies is the acquisition of suitable material for investigation. As a result, literature dealing with aging effects on the endocrines is quite meager, exceptionally so for fishes. Interpretation of changes in pituitary morphology accompanying the onset of senility is difficult unless structural and functional changes in target organs and determination of circulating levels of hormones can be evaluated, which was not attempted in this study. Stating that a cell is "active" or "inactive," "granulating" or "degranulating," is often a questionable procedure unless the above parameters are taken into consideration.

In what seems to be the only report dealing with aging effects on the fish testis, Rasquin & Hafter (1951) called attention to the similarity of the changes they found in *Astyanax* to those described in other vertebrates. They described connective tissue infiltration and formation of "concretions" in the lobules and spermatic ducts. Of special interest is their finding of areas of active spermatogenesis even in the testes of a six-year-old fish. Generally, the reproductive capacity of live-bearing poeciliids decreases with age (Gerking, 1959). Baker-Cohen (1961) found that after the attainment of maturity there is a marked decline in thyroid activity in the platyfish. Both thyroid and testis are under the control of the pituitary, and the changes accompanying senility might therefore be reflected in the cyanophil population of that gland. In female platyfish, both the peripheral and central cyanophils of the meso-adenohypophysis exhibit distinct changes associated with increased age.

The report presented here for pituitaries from aging female platyfish shows many similarities (*e.g.*, increased chromophobia, decreased acidophilia, vacuolation of some cyanophilic elements) to the changes reported for the hamster

(Spagnoli & Charipper, 1955) and man (Kinsell, 1961). The paucity of related information essential to evaluate these morphological changes is well illustrated by Kinsell's conclusion that "no definite functional interpretation of histologic changes is possible at this time."

E. Pituitaries in Pregnant Platyfish

The marked enlargement of the meso-adenohypophysis in gravid platyfish is reflected in the increased size of the total pituitary gland. The dimensions reported by Vervoort (1957) for the hypophysis in the pregnant guppy, are comparatively smaller (200 × 100 micra for the guppy in contrast to 543.2 × 213.3 micra).

In the gravid platyfish, glandular increase is associated with hypertrophy, hyperplasia, and degranulation of the peripheral cyanophils and a marked increase in number, but not in size, of the red-orange acidophils of the dorsal meso-adenohypophysis. A series of projected drawings of serial sections by Baker-Cohen (1961) of the pituitary of an untreated gravid platyfish, also shows an enlarged glandular area, especially along the ventral surface. This enlarged zone can be attributed to the hypertrophy and hyperplasia of the peripheral cyanophils characteristic of gravid platyfish. Sokol (1961) reported an inverse relationship between the acidophils and ventral cyanophils of the meso-adenohypophysis of the guppy. In the gravid guppy, near the time of parturition and during the early days of a subsequent pregnancy, the ventral cyanophils are devoid of granules, and the meso-adenohypophysial acidophils exhibit maximum activity, that is, they are highly granular. If this is true in platyfish, it would seem that the majority of the animals in this study were in either the early or the late stages of pregnancy.

It appears probable that prolactin is synthesized by acidophils (Barrnett, Siperstein & Josimovich, 1956; Riddle, 1963). Friedgood & Dawson (1938, 1940) have shown that carminophils fluctuate in number during the reproduction cycle of rabbits and cats and that this is associated with pregnancy and lactation. In mammals, prolactin or luteotrophin (LTH) is generally considered to be responsible for the initiation of progesterone secretion by the corpus luteum. In the platyfish, the appearance of increased numbers of acidophils during pregnancy is somewhat of an enigma in light of the conclusion of Pickford & Atz (1957, p. 221) that "there is no indisputable evidence for the endocrine nature of the so-called corpus luteum of the teleost." Nevertheless, Egami & Ishii (1962) have demonstrated that prolactin is effective in maintaining gestation in two genera of fish, *Gambusia* and *Ditrema*. Perhaps prolactin works synergistically in the regulation of gonadotrophins and sex hormones in fish as Pickford (1959) suggested. At this level of the phylogenetic scale, prolactin may also have different physiological roles, as for example in pigment formation (Pickford & Kosto, 1957), parental behavior (Riddle, 1963), and in mucus production in the skin of post-spawning, parental *Symphysodon discus* (Egami & Ishii, 1962). Of course there is also the possibility that no relationship exists between the acidophil hyperplasia and prolactin elaboration and secretion in the platyfish.

F. Pituitaries of Thyroid Tumorous Platyfish

In response to an iodine deficient environment (*i.e.*, distilled water), platyfish develop a goitrous condition. This "tumorous state" can be mitigated simply by the addition of iodine to the diet of these fish (Baker, 1958b). The thyroid hyperplasia is undoubtedly brought about by increased thyrotrophin (TSH) release in response to a deficiency of thyroxine in the blood stream. This manifestation is comparable to that achieved when thiouracil is administered (Turner, 1960; Goldsmith *et al.*, 1944).

The pituitary cytology of platyfish with thyroid tumors presumably reflects this marked release of TSH from the pituitary. Only the cyanophils in the mid-region of the meso-adenohypophysis exhibit a marked degranulation, loss of PAS- and aldehyde fuchsin-positive material, and a dramatic increase in cell number and size, including an almost threefold increase in cellular dimensions. No vacuolation comparable to what is found in the thyroidectomy cells of the rat has ever been observed. On the other hand, the peripheral cyanophils, as a result of a decrease in their volume, appear to be much denser. The appearance of the peripheral cyanophil is similar to the change in the gonadotrophs that accompanies thyrotroph degranulation in thiourea-treated and ACTH- and cortisone-treated *Astyanax* (Atz, 1953).

Our observations are in agreement with the description presented by Öztan (1961). Degranulation of specific cyanophils in response to thiourea treatment has also been recorded for *Brachydanio rerio* (Scott, 1953), *Phoxinus phoxinus* (Barrington & Matty, 1955), *Dentex dentex* (Leloup & Olivereau, 1950) and *Poecilia reticulata* (Sokol, 1955). Radioiodine (I^{131}) administered to *Anguilla anguilla* also evoked a characteristic hyperplasia, hypertrophy and deganulation of cyanophils (Olivereau, 1963). Honma & Murakawa (1955) reported that in thiourea-treated salmon larvae, an enlargement of the cells in the transitional lobe occurs, but they did not specify the cell types affected. The significance of the loss of aldehyde fuchsin- and

PAS-positive granules, as seen in the platyfish, has been discussed for the rat. A rising level of TSH in the blood is inversely related to the loss of aldehyde fuchsin-positive (Del Conte & Stux, 1955) or PAS-positive (Purves & Griesbach, 1951a) material. On the other hand, there are reports on related phenomena that are difficult to reconcile with the present investigation of thyroid-tumor bearing platyfish and the large body of supporting information that is available. For example, Rasquin (1949) found no change in basophil counts of thiourea-treated *Astyanax*, Stolk (1956a, b, c) reported no cytological changes in three genera of thyroid tumorous fish, although he does note an increase in glandular area, and Baker-Cohen (1961) found no cellular increase in the pituitaries of "severely hypothyroid or completely athyroid fish" (radioiodine treated young fish). In the last instance, Baker-Cohen suggested that this may be an example of an exhausted pituitary.

In her report, Baker-Cohen (1961) described a "prominent overgrowth of pale intermediate basophiles" in radioiodine-treated adult platyfish. In addition, she reported that in several fish with thyroid hypertrophy, the pituitary structure had changed; there was "a marked overgrowth of the paler intermediate basophiles, so that these overlapped the darker anterior basophiles ventrally, causing the appearance of a sharp line of demarcation in the anterior region and a distortion of the usually round cross-section of the hypophysis at that point." Although evaluation of this description is difficult because of the terminology used for location and cell types, the cellular "overgrowth" Baker-Cohen described might be the result of the same hypertrophy and hyperplasia of the central meso-adenohypophysial cyanophils found in goitrous fish of the present study.

G. Effects of Castration on the Pituitary of Male Platyfish

Information on the effects of castration on teleost pituitaries is conspicuously absent. The only report involving surgical castration appears to be Sokol's (1955) study of ovariectomized guppies, but she fails to give any cytological detail. Atz (1953) has presented a cytological description of the pituitary in physiological castrates. *i.e.*, *Astyanax* that were reared in the dark.

In surgically castrated male platyfish, the cyanophils of the peripheral meso-adenohypophysis contain vacuoles, exhibit degranulation, are hypertrophied, and have increased in number. Typical "castration cells", although characteristically present, are not numerous. Severinghaus (1939) indicated that marked hyperplasia is a more prominent feature of the pituitaries of castrate mammals than the presence of signet-ring cells. This also holds for the pituitaries of surgically castrated male platyfish with their paucity of "castrate cells" and prominent cyanophil hyperplasia and hypertrophy. The description of the castration cell in *Astyanax* (Atz, 1953) and the rat (Purves & Griesbach, 1951a) does not differ essentially from the one described in castrated male platyfish.

In the platyfish, cyanophils that respond strikingly to castration are confined to the peripheral area of the meso-adenohypophysis as also reported for the castrate female guppy (Sokol, 1955). In *Astyanax*, this cell type is found in the central area in addition to the peripheral location (Atz, 1953). In reviews of the literature on pituitary cytology in teleosts, Olivereau (1962b, 1963) indicated that invariably the cells that are affected by reproductive cycles and functional variations in the gonads are located in a region that corresponds to the meso-adenohypophysis. Öztan (1963) reported that in sterile hybrids between platyfish and swordtails, "peculiar adenohypophyseal basophils" are degranulated, smaller, and fewer in number, but unfortunately she did not give the location of these cells. In an earlier report, however, Öztan (1961) cited unpublished data and suggested that "type 3" cells (which correspond to the peripheral cyanophils of the meso-adenohypophysis, p. 221) may be involved in gonadotrophic function.

The cyanophils of the central meso-adenohypophysis that change vividly in platyfish with thyroid neoplasms also appear to be altered in castrate males, *i.e.*, they increase somewhat in cell and nuclear dimensions and exhibit some degranulation. It has been demonstrated that the thyroid gland generally plays an important role in gonadal development in many animals and that interference with thyroid function may be reflected in the delay of sexual maturity (Barrington & Matty, 1952; Baker-Cohen, 1961) or the development of secondary sex characters (Goldsmith *et al.*, 1944; Nigrelli, Goldsmith & Charipper, 1946). Perhaps the cytological changes of the central cyanophils, which the present study has shown to be responsive to iodine deficiencies, may be a manifestation of a thyroid-gonad syndrome.

The inability to identify cells undergoing mitosis is puzzling in pituitary glands that exhibit marked hyperplasia, *e.g.*, in castrated and goitrous platyfish. Perhaps the proliferation of cellular elements exhibits a periodicity such as that frequently encountered in proliferating plant and animal tissues, *e.g.*, regenerating rat liver and onion root meristematic tissue, and the time chosen for fixation may not have coincided with cell division. This would imply that all investigators have killed and fixed their specimens at a time of minimal cell division because mitotic figures have been so infrequently reported in the pituitary gland. Van Mullem (1958) speculated on the possibility that pituitary cells may divide amitotically in addition to a normal mitotic process. His hypothesis, however, is based solely on the frequency of variably shaped nuclei (*e.g.*, bean-shaped, indented) that he encountered.

H. Pituitaries in Fish With Melanotic Tumors

Mac Intyre & Baker-Cohen (1961) described the pituitary of a spike-tailed platyfish *(Xiphophorus variatus xiphidium)* that exhibited both melanoma and thyroid tumor. Although the hypophysis in this fish "was not sectioned or stained in a manner suitable for the demonstration of cell types," these investigators discovered abnormalities described as follows:

> "In the anterior part of the gland, the vertical cross-section was much elongated; the elongation was made up of a homogeneous mass of light staining basophils. Eosinophils were not delineated by the staining methods employed. Normally, at that cross-sectional level, the pituitary is round and chiefly composed of darkly staining basophils; the paler cells appear in small numbers along the ventral border and increase somewhat in the posterior direction, as the neural portion of the hypophysis appears. In the tumorous fish, the anterior basophilic overgrowth also continued along the entire length of the pituitary, making the entire organ misshapen."

Mac Intyre & Baker-Cohen stated that this overgrowth of "paler basophilic elements" was also seen in *X. maculatus* with goitrous thyroids or with regenerating thyroid tissue after radioiodine administration. Although the present work did not include a specimen with both pathological conditions, it is difficult to apply this description to either our melanomatous fish or fish with thyroid tumors. In the present study, thyroid tumorous fish were found to possess pituitaries with hyperplasia and hypertrophy of the central meso-adenohypophysial cyanophils, while in pituitaries from melanomatous fish, the acidophil population was most affected. The inability of Mac Intyre & Baker-Cohen to see a change in the acidophil population can be attributed to the fact that they were unable to demonstrate acidophils. In another spike-tailed platyfish with extreme melanosis, but no thyroidal neoplasm, Mac Intyre & Baker-Cohen (1961) failed to note any structural differences in the pituitary. This fish had been fixed by "slow formalin fixation."

Hypertrophy of the pro-adenohypophysial acidophils and marked hyperplasia of the meso-adenohypophysial acidophils are the most striking features of pituitary glands in melanoma-bearing platyfish. Less consistent is the hypertrophy (statistically insignificant) of the cyanophils of the meta-adenohypophysis. Levenstein's (1939) differential counts revealed that a greater number of acidophils are present in the transitional lobe (meso-adenohypophysis) of the black moor goldfish than in the common goldfish. He suggested that the somatic differences between these two varieties (*i.e.*, abnormal growth pattern, telescopic eyes and black pigmentation) may be reflected in the difference in pituitary chromophil cell counts. The association of increased acidophilia of the "transitional lobe" with black-pigmented goldfish is interesting in the light of the enhanced acidophilia found in the hypophyses of platyfish with melanoma.

Chavin (1956) indicated changes in the pituitary cytology of the goldfish after various hormonal treatments designed to analyze the role of these substances in melanogenesis, but he did not discuss the significance of the cytological changes. He found that ACTH stimulates melanogenesis in both hypophysectomized and intact goldfish. The administration of ACTH alone, or in conjunction with intermedin, causes the acidophils of the transitional lobe (meso-adenohypophysis) to enlarge, become densely granulated, and stain deep red (with Masson stain); there is no variation in the pro-, meta-, or neurohypophysis (our terminology) with this treatment. Chavin (1959) found that in xanthic goldfish, surgical removal of the pars tuberalis (pro-adenohypophysis) and pars distalis (meso-adenohypophysis) inhibits melanogenesis. In contrast, extirpation of the pars intermedia (meta-adenohypophysis) has no effect.

Pickford (1956) and Pickford & Kosto (1957), however, have demonstrated that prolactin is the pituitary hormone necessary for melanogenesis in *Fundulus heteroclitus.* They found that sheep prolactin alone will increase melanin in pre-existing melanophores; when this hormone is administered in conjunction with intermedin, both an increase in melanin formation and the appearance of new melanophores occurs. In contrast, no melanogenesis follows the administration of ACTH. A later report, with *in vivo* observations and *in vitro* biochemical assays, confirmed the latter observation and indicated that the action of ACTH resembles intermedin (Kosto, Pickford & Foster, 1959). If, as claimed by these authors, prolactin is essential for melanogenesis in *Fundulus,* our observation that the pituitaries of melanomatous and gravid platyfish exhibit a hyperplasia of the meso-adenohypophysial acidophils, may indicate that these acidophils are, (1) involved in both pregnancy and melanogenesis, and (2) that the secretion of these cells is prolactin.

Because a species difference in the hormonal control of melanogenesis could very well be possible, an investigation of this process in platyfish would undoubtedly yield useful comparative information.

The foregoing might suggest that there are two physiologically distinct classes of acidophils in the meso-adenohypophysis, *viz.*, those concerned with both gestation and melanization and others associated with growth hormone. However, morphologically only one type of acidophil was detected in the meso-adenohypophysis. Perhaps this differentiation awaits a more sophisticated probe. It is also conceivable that one cell type may elaborate more than one active substance.

The possible existence of a melanophore-concentrating hormone originating in the pro-adenohypophysis (Pickford & Atz, 1957; p. 35-36) may partially explain the hypertrophy of pro-adenohypophysial acidophils in melanoma-bearing platyfish. It is also possible that another hormone from the pro-adenohypophysis may play an as yet unelucidated role in the melanotic and melanomatous hybrids.

I. Distribution of Thyrotrophs and Gonadotrophs in the Platyfish Pituitary and an Evaluation of the Periodic Acid-Schiff and Aldehyde Fuchsin Tests

From an analysis of PAS- and aldehyde fuchsin-stained specimens the following generalizations can be made:

(1) The two classes of meso-adenohypophysial cyanophils cannot be distinguished by their response to these techniques; thus gonadotrophs and thyrotrophs cannot be demarcated. With such differential methods, Purves & Griesbach (1951a, b, c) and Halmi (1952) were able to delineate two classes of cyanophils in mammalian pituitaries and Barrington & Matty (1955) accomplished this in the minnow. Atz (1953) and Sokol (1955), however, could not separate two classes of cyanophils on the basis of differential staining responses.

(2) The intensity of the reaction with these techniques corresponds to the degree of cyanophilia.

(3) The application of aldehyde fuchsin is an excellent means of demonstrating neurosecretory granules and Herring bodies in the neurohypophysis.

The identification of thyrotrophs in the central part of the meso-adenohypophysis and gonadotrophs in the peripheral zone has been possible on the basis of the following indirect evidence presented in this report:

(a) Marked hypertrophy and degranulation of only the central cyanophils occur in response to a deficient iodine environment.

(b) Conspicuous hypertrophy, hyperplasia, and vacuolation of the peripheral cyanophils distinguish pituitaries of castrate males.

(c) Striking changes take place in the peripheral cyanophils of gravid platyfish.

(d) The presence of dorsal cyanophils early in the postpartum development of *X. maculatus* at the time when a functional thyroid is already present. The appearance of a peripheral cyanophilic layer later in postpartum development synchronous with gonadal maturation.

Summary

1. The pituitary gland of *Xiphophorus maculatus* is composed of four regions: pro-adenohypophysis, meso-adenohypophysis, meta-adenohypophysis and neurohypophysis.

2. The pro-adenohypophysis occupies the most anterior portion of the gland and is populated by carminophilic acidophils, which predominate, and chromophobes. A peninsula of chromophobic cells and a connective tissue septum form the posterior boundary of the pro-adenohypophysis.

3. Two regions in the meso-adenohypopohysis are distinguishable: a central portion composed of orange acidophils (with Heidenhain's azan) and round cyanophils that are grouped in islets formed by penetrating strands of neurohypophysial tissue, and a peripheral zone of cyanophils that vary in shape and size. Small numbers of chromophobes are dispersed in this region.

4. The meta-adenohypophysis, which comprises the posterior part of the gland, is made up of round or oval cyanophils and a second cell type that is generally spindle-shaped and agranular.

5. The neurohypophysis is characterized by a highly branched network of fibroid material, large nuclei, neurosecretory granules and Herring bodies. Its ramifications penetrate all the glandular portions to varying degrees. Ependyma cells border on the infundibular cavity and hypophysial recess.

6. No distinction can be made between the two classes of meso-adenohypophysial cyanophils based on a differential response to PAS and aldehyde fuchsin. The intensity of the reaction with these techniques corresponds to the degree of cyanophilia.

7. In one-week-old fish, the neurohypophysis and adenohypophysis occupy about equal areas. Except for the cyanophils of the peripheral meso-adenohypophysis, all cell types that can be identified in the pituitaries of mature specimens are present in one-week-old fish. Cyanophils of the peripheral meso-adenohypophysis are not evident until six weeks postpartum; by eight weeks they have increased in number and contain more granules. The significance of the differential appearance of the two classes of cyanophils and the possible role of the various cell types in the early development of platyfish is discussed.

8. In the pituitary of aging female platyfish one finds: an increased chromophobia, hypertrophy and degranulation of the peripheral cyanophils, a decrease in cell and nuclear size of the central cyanophils and an apparent increase of neurohypophysial tissue. There also appears to be a change in hypophysial shape with senility.

9. Pituitaries of fish with heterotopic thyroid tumors exhibit a pronounced protuberance of the meso-adenohypophysis and degranulation, hypertrophy and hyperplasia of the central cyanophils. The cyanophils of the peripheral and protuberant areas are smaller, hyperchromic and dispersed.

10. Hyperplasia, hypertrophy and prominent vacuolation of the peripheral cyanophils characterize the pituitaries of surgically castrated males. A description of "castration cells" as they appear in platyfish is provided. Central cyanophils are irregularly shaped and slightly enlarged. An increase in the dimensions of the meso-adenohypophysis is reflected in an increase of the total gland volume.

11. The hypophysis of gravid platyfish can be identified by a prominent increase in the width of the peripheral layer of cyanophils and hyperplasia of the central meso-adenohypophysial acidophils. Peripheral cyanophils have increased in number and size. Their cytoplasm is hyaline in appearance and contains many spherical droplets and clear vacuoles.

12. Hyperplasia of the meso-adenohypophysial acidophils and hypertrophy of the pro-adenohypophysial acidophils distinguish the pituitary of melanomatous fish. Cellular elements of the meta-adenohypophysis are insignificantly enlarged. The significance of the hyperplasia of the meso-adenohypophysial acidophils in both melanomatous and gravid platyfish is discussed in the light of recent theories of hormonal control of melanogenesis.

13. The evidence is discussed that permits identification of thyrotrophs in the central meso-adenohypophysis and gonadotrophs in the peripheral meso-adenohypophysis of the platyfish pituitary.

14. Data are given for glandular and cellular dimensions and differential cell counts reported for most classes of pituitaries investigated.

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EXPLANATION OF THE PLATES

Plate I

Fig. 1. Sagittal section of a pituitary from a sexually mature female. Dark area at the right is the pro-adenohypophysis with its characteristic abundance of carminophilic acidophils. Dark gray area at the extreme left is the meta-adenohypophysis. Between these two regions is the meso-adenohypophysis. The neurohypophysis is in the mid-dorsal position. Heidenhain's azan, formol-sublimate. 190×.

Fig. 2. High power view of carminophilic acidophils and adjacent cells (gray) of the wedge area in the pro-adenohypophysis. Light area at the extreme left is a portion of the neurohypophysis. 1025×.

Fig. 3. High power view of the central meso-adenohypophysis. Note islets of cells formed by pervading neurohypophysial tissue. 1025×.

Fig. 4. The cell types of the meta-adenohypophysis at high magnification. Note adjacent neurohypophysial tissue at the right of the picture. 1240×.

Plate II

Fig. 1. Sagittal section of a pituitary from a one-week-old platyfish. Anterior end is at the right. Chrome alum fixed, Heidenhain's azan stained. 460×.

Fig. 2. Pituitary of an eight-week-old fish; sagittal section. Compare with fig. 1. Acidophils are black; cyanophils are gray. In this photograph the anterior end is at the left. Arrow points to ventral cyanophil layer that is absent in one-week-old glands. 300×.

Fig. 3. High power view (sagittal) of the meso-adenohypophysis of the gland represented in fig. 1. Acidophils are black; cyanophils are lighter. Arrows in figs. 1 & 3 point to similar areas of the meso-adenohypophysis. 1240×.

Fig. 4. Higher magnification of the meso-adenohypophysis of gland depicted in fig. 2. Here cyanophils are black and acidophils are lighter. 1240×.

Plate III

Fig. 1. Aldehyde fuchsin response (black) in a gland of a sexually mature platyfish. Dark area towards the right of the gland is neurosecretory material in the neurohypophysis. Formol-sublimate. 170×.

Fig. 2. Distribution of PAS-positive material (black) in a sagittal section of a pituitary gland from a sexually mature fish. Compare with adjacent section represented in fig. 1. Formol-sublimate. 170×.

Fig. 3. Pituitary from a castrated male platyfish, in sagittal section. Note marked increase in ventral meso-adenohypophysial cyanophil layer (lighter lower area in mid-region of the gland). Helly fixed, azan stained. 160×.

Fig. 4. High power view of ventral cyanophil cell layer of a pituitary from a castrated male platyfish. Note "castration cells." 1240×.

Plate IV

Fig. 1. Sagittal section of a pituitary from a 28-month-old female platyfish. Note large chromophobic area (gray) in pro-adenohypophysis (left side). Heidenhain's azan following Helly's fixative. 230×.

Fig. 2. Mid-sagittal section through a pituitary from a fish with a heterotopic thyroid tumor. Note protuberance of meso-adenohypophysis (central portion). Dark area at extreme left is pro-adenohypophysis. 170×.

Fig. 3. High power of the mid-region of gland in fig. 2. Note large cells with loss of granules in central area and smaller, darker, and separated cells at periphery (bottom of picture). Chrome alum fixed, azan stained. 580×.

Fig. 4. Similar region as fig. 3 from another thyroid tumor-bearing platyfish. This section, however, was stained with aldehyde fuchsin. 460×.

Plate V

Fig. 1. Sagittal section of a hypophysis from a gravid fish. Note increased ventral meso-adenohypophysial cyanophil cell layer (lower mid-region). Note, too, many dark cells (acidophils) dorsal to this cyanophil layer. 170×.

Fig. 2. Higher magnification of gland from a gravid fish. Here hyperplasia of meso-adenohypophysial acidophils (black) and increased ventral cyanophil layer (light) is quite evident. 510×.

Fig. 3. Mid-sagittal section of a non-melanotic platyfish-swordtail hybrid pituitary. 230×.

Fig. 4. Pituitary of hybrid fish with a melanoma. Note especially the marked acidophil hyperplasia in the central (meso-adenohypophysis) region and hypertrophied acidophils and their nuclei of the pro-adenohypophysis (right side). Compare with fig. 3. 270×.

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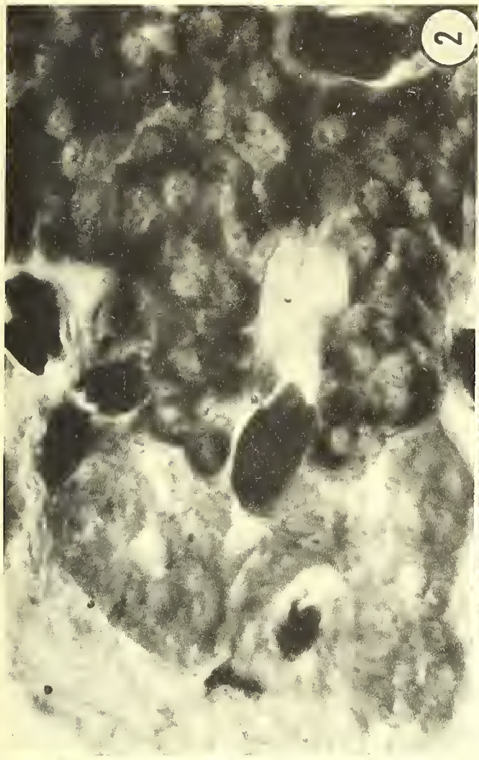

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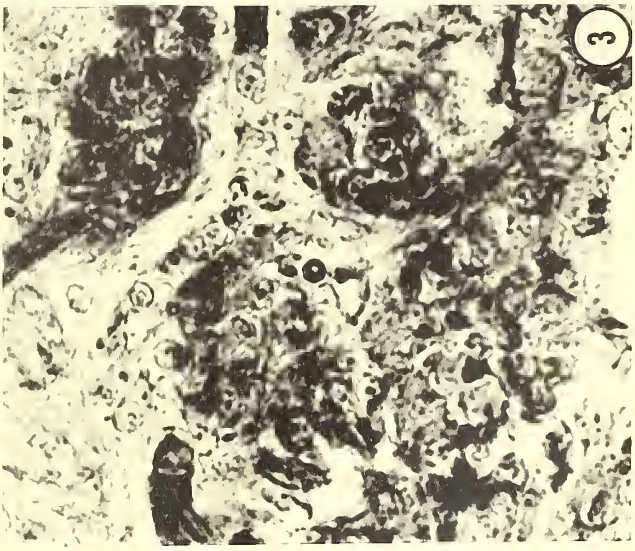

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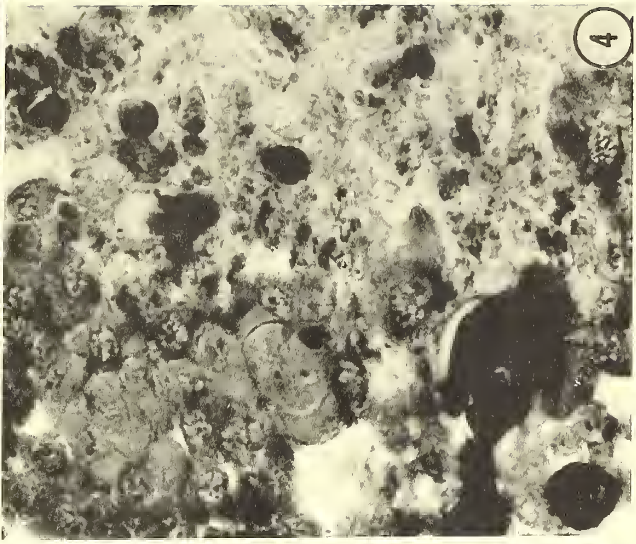

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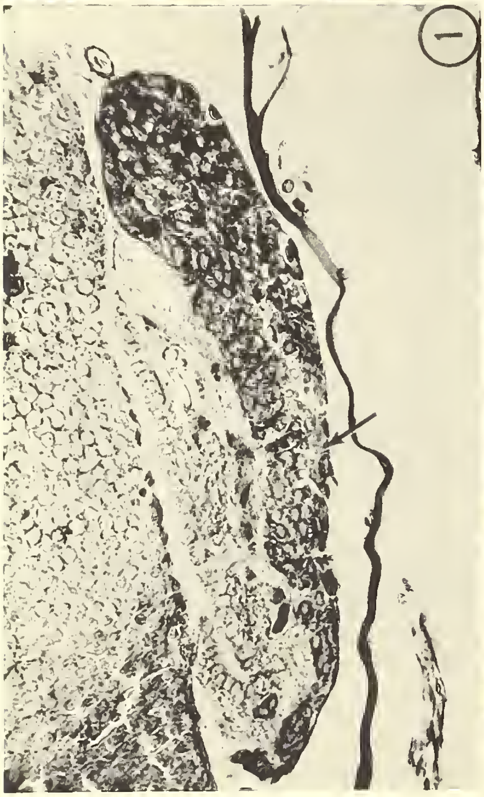
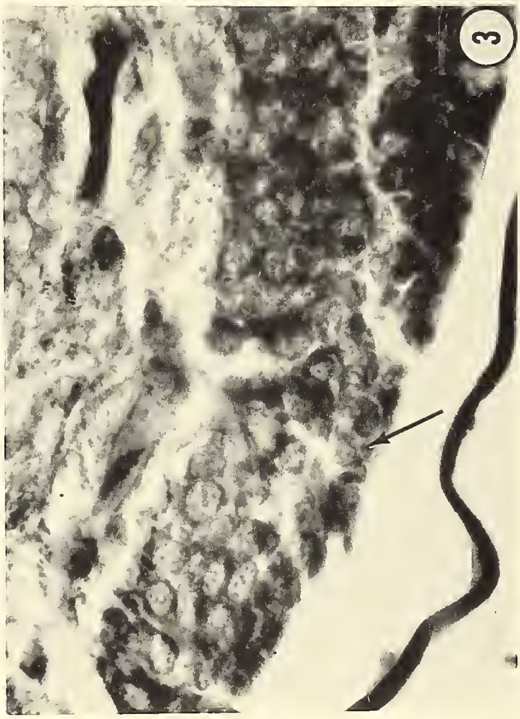
STUDIES ON THE PITUITARY GLAND OF XIPHOPHORUS MACULATUS (THE PLATYFISH)





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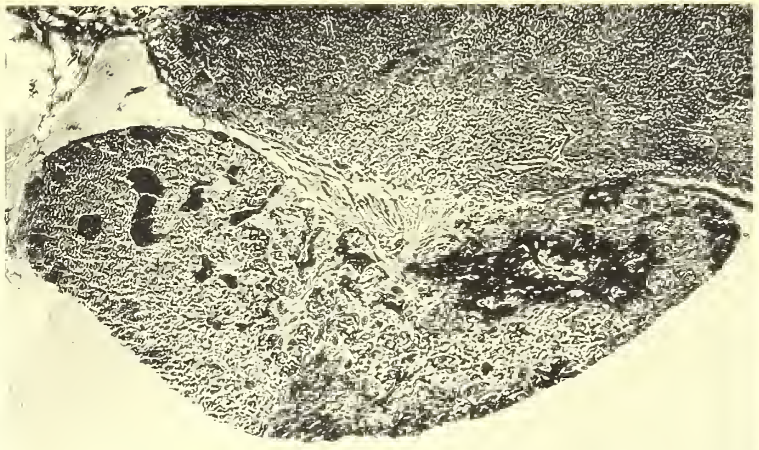

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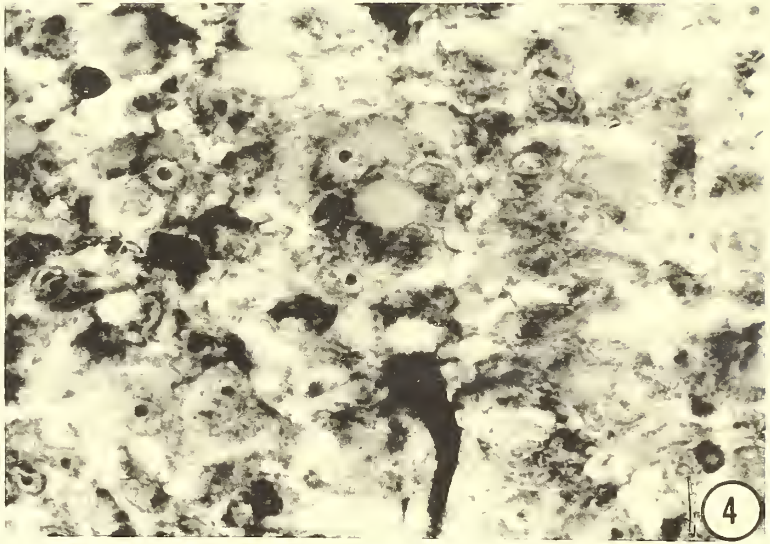

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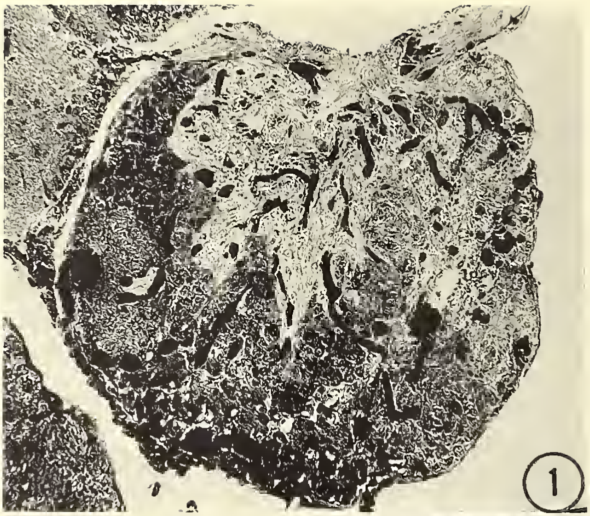
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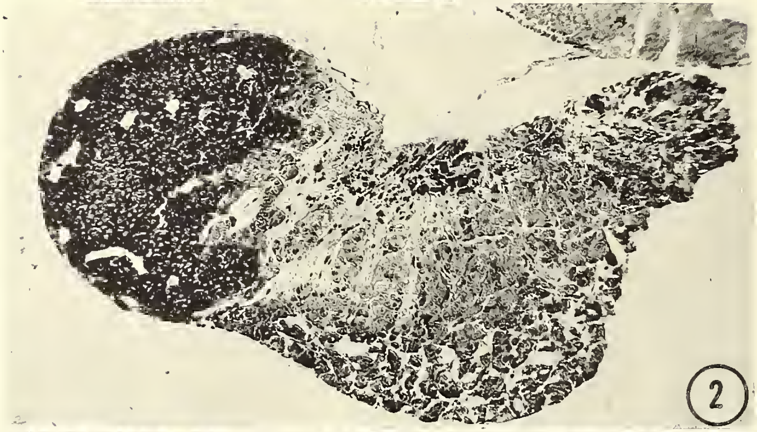
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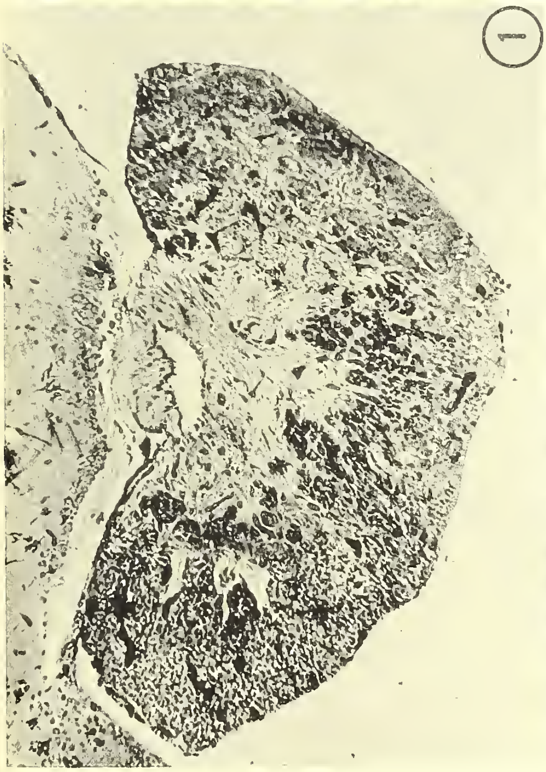

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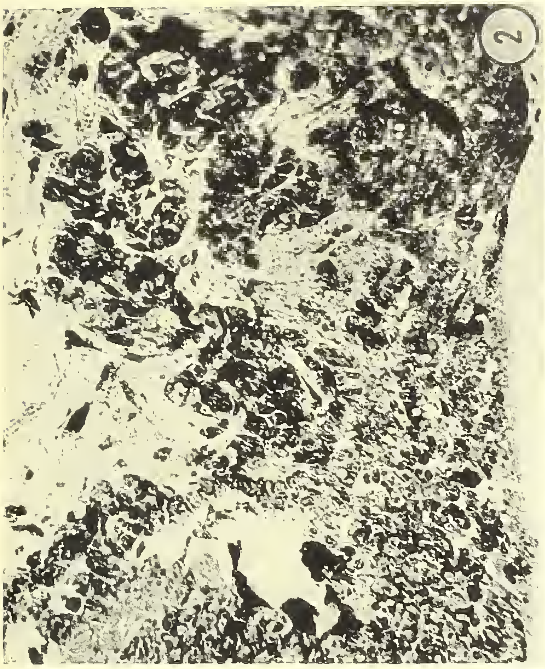

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STUDIES ON THE PITUITARY GLAND OF XIPHOPHORUS MACULATUS (THE PLATYFISH)

15

The Geographical Distribution of the Color-pattern Components of *Heliconius erato* and *Heliconius melpomene* with Genetical Evidence for the Systematic Relationship between the Two Species[1,2]

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(Plates I & II; Map 1; Text-figs. 1-15)

[This paper is a contribution from the William Beebe Tropical Research Station of the New York Zoological Society at Simla, Arima Valley, Trinidad, West Indies. The Station was founded in 1950 by the Zoological Society's Department of Tropical Research, under Dr. Beebe's direction. It comprises 250 acres in the middle of the Northern Range, which includes large stretches of government forest reserves. The altitude of the research area is 500 to 1,800 feet, with an annual rainfall of more than 100 inches.

[For further ecological details of meteorology and biotic zones see "Introduction to the Ecology of the Arima Valley, Trinidad, B.W.I." by William Beebe, Zoologica, 1952, Vol. 37, No. 13, pp. 157-184].

CONTENTS

I. INTRODUCTION AND ACKNOWLEDGMENTS

THE most important early attempt to rationalize the diversity of color and pattern within the genus *Heliconius* was by Eltringham (1916), who based his conclusions upon macroscopic wing characters and an examination of the male genital valves. Recent genetical studies by Turner & Crane (1962) and Sheppard (1963) on material collected from Surinam and Trinidad, have shown that some of the more conspicuous components of the color-patterns of *Heliconius erato* and *Heliconius melpomene* are genetically linked and are capable of occurring polymorphically in both species.

Through funds supplied by the National Science Foundation, it was possible in 1963 to make field observations in two localities in Colombia and to observe and collect breeding material from several localities in eastern and western Ecuador.

The objectives of this project were, firstly, to test conspecificity within *H. erato* and *H. melpomene* by breeding; secondly, to confirm that similar color-pattern components occur sympatrically in both species so as to produce what appear to be mimetic pairs; and, thirdly, to observe the phenotypic characters of laboratory-bred heterozygotes and to obtain linkage data to test the closeness of the systematic relationship between the two species.

Thanks are due to the National Science Foundation for the financing of the field trips and technical assistance, to Jocelyn Crane for the promotion of the work, to Julie Emsley for taking charge of the laboratory rearings and draw-

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[1] Contribution No. 1059, Department of Tropical Research, New York Zoological Society.

[2] This study has been supported by the National Science Foundation (G 21071).

ings and to Dr. E. W. Schmidt-Mumm[3] for considerable help and advice in Colombia. Dr. P. M. Sheppard, of the University of Liverpool, should be singled out for particular thanks as he has contributed a generous amount of his time to the evaluation of the conclusions to be drawn from the data and in statistically processing the data upon which the Text-figure histograms have been drawn.

II. Materials and Methods

Three separate visits were made to Ecuador in 1963, from April 26 to May 15, from July 2 to 17, and from August 20 to 28. On the first trip the intention was to follow the system used on earlier trips to Surinam and collect eggs, larvae and pupae of local forms of *H. melpomene* and *H. erato* and return to Trinidad with the live immature material by a single-day jet flight from Guayaquil. However, great difficulty was experienced in keeping larvae alive during travelling in Ecuador because of mechanical and climatic disturbances, and because of the difficulty of providing a continuous supply of fresh food. All the larvae and pupae that were collected died, either in transit or later in Trinidad. Non-acceptance of Trinidadian *Passiflora* by larvae reared on exotic species also contributed to the extinction of the material. However, on the last day of the first trip, near Bucay, western Ecuador, a single male of *H. melpomene cytherus* and some females of *H. erato cyrbius* were caged in a box containing a pad soaked in a solution of sugar in water, and survived the journey back to Trinidad to form the foundation of the breeding stocks. These females which were already gravid, showed no hesitation in laying their eggs on the same species of *Passiflora* used by their Trinidadian counterparts and furthermore rejected any other species of *Passiflora* offered to them. While being brought back from the collecting locality by road, the box containing the adults was cooled by the intermittent application of external ice packs. On both subsequent visits, adults were caught in the field with a net, kept in cubical eighteen-inch gauze cages and fed on local flowers or a solution of honey in water. During long distance airplane flights the butterflies were transferred to small translucent paper envelopes and loosely packed in boxes with tissue paper. Using this method, mortality was less than 10%.

The normal mating and laboratory techniques have already been summarized by Turner & Crane (1962), but as an additional precaution against the particularly high incidence of virus disease, all the glassware was sterilized in an oven immediately before use and all food material and cotton wool pads were rinsed in a 5% solution of Milton, which is 1% sodium hypochlorite and 16% sodium chloride. Even so, mortality became so high that only by repeatedly changing the location of the laboratory rearing was complete extinction delayed, and ultimately the genetical experiments had to be abandoned. Mortality data are presented in the appendix.

The distributional data are based upon the examination of the collections of the British Museum (Nat. Hist.) at London and Tring, the Hope Department of Entomology at Oxford, the Paris Museum of Natural History and a portion of the collection of the American Museum of Natural History, as well as upon advice from Dr. E. W. Schmidt-Mumm on the Colombian fauna, and personal experiences in Ecuador and Colombia. Thanks are offered to the trustees and staff of the above institutions for study facilities.

III. Description of the Wild Populations

The geographic and polymorphic variation in these two species is so diverse that to describe the distribution of the named forms unnecessarily complicates the task. Instead, the individual characters will be described and their distribution plotted on maps. By reference to the data in Table I, the characters may be related to the published names, of which those described prior to 1929 have been catalogued by Neustetter.

Confined to the neotropics, *melpomene* and *erato* probably occur in all areas where tropical or subtropical vegetation contains suitable species of the larval food plant *Passiflora*, and as far as can be ascertained the ranges of the two species grossly coincide (Map 1). The only localities where *erato* seems to occur on its own are in the valley of the Cauca river in Colombia, north of Nicaragua, along the east coast of Brazil and over its southern limits in Paraguay. *Melpomene*, as judged by museum data, is less common than *erato* in the south, and considering its habits of frequenting the more shady habitats it may have been overlooked at the southern extremity of its range, for even records of *erato* are very few beyond 25° South.

There is evidence that *melpomene* occurs in the absence of *erato* at about 1,250 meters in the eastern Ecuadorian Andes.

Within the latitudinal limits of their range, the principal regions where both species are absent are above 1,300 meters in the Andean, Guianian and Brazilian highlands, and west of the Andes south of Ecuador. It also seems unlikely that they colonize the tropical grasslands which comprise the llanos of Venezuela and the campos of Brazil. There are only sparse records from the area in southeastern Peru that is

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[3] Dr. E. W. Schmidt-Mumm, Optometra, Bogotá, Colombia.

MAP 1. The geographical distribution of *Heliconius erato* and *Heliconius melpomene*. The black shaded areas are where neither species is known to occur, the darker shaded areas are where their distribution coincides, and the lighter shaded areas are where *erato* appears to occur on its own.

drained by the Madeira river, but this may be due only to inaccessibility, for it is flooded to a depth of a meter for at least two months of each year. Within their gross range it is likely that there are many areas where the ecological conditions are unsuitable, but our knowledge is focused around the commercial centers and waterways, for communications in South America are still very restricted. In the late nineteenth and early twentieth centuries, which is the period during which the bulk of the world's museum material was collected, communications were limited to river steamers, canoes and mule trains. Even now, motorized transport is at such a premium outside the cities of South America that sites for collecting are still influenced by availability as well as zoological reasoning.

Much of the early material is inadequately labelled. For example, "Ecuador" covers both sides of the Andes, and "west Ecuador" may still be a locality east of the Andes, for prior to 1942 Ecuador extended eastward as far as Brazil, and the eastern slopes of the Andes were still relatively "west." Ambarto and Quito are large towns lying between the crests of the twin mountain ranges, and such a locality label does not indicate whether the insect was taken on the western or eastern slopes, for both are far too high to be actual sites of collection. The same remarks apply also to Bogotá. "Amazonas" is a frequent label name, but not only is Amazonas-Brazil a vast area but there are "Amazonas" provinces in Venezuela, Ecuador and Bolivia as well. "Five days north of Cochabamba" is more useful, for in 1899 a journey was presumably accomplished either on foot or on mules. In some taxonomic groups data such as "eastern Ecuador" would be very useful, but in these species there are so many localized pockets of distinct forms that one needs to have such detailed information as "40 km. from Puyo on Puyo-Napo road, 800 m." Our earlier ideas on the prevalence of polymorphism have been exaggerated by the lack of precision in data from the eastern slopes of the Andes.

The color-patterns of both species are composed of a small number of similar components which are figured semi-diagrammatically on Plate I and described in detail below.

Heliconius erato (Linnaeus 1758)

GROUND-COLOR (Pl. I, figs. 1-8). In all specimens studied, the ventral surface of both wings is a dull and uniform milk-chocolate brown (Pl. I, fig. 7), but the dorsal surface, though similar on both fore and hind wings, varies geographically from a brilliant structural blue iridescence (Pl. I, figs. 2 & 4), to a matt black (Pl. I, figs. 1, 3, 5). The most brilliant blue is exhibited by specimens from western Ecuador (*cyrbius* Godart 1819) and western Colombia (*venus* Staudinger 1882), whence there is a cline of decreasing blueness which forks in northern Colombia to Panama and Central America on the one hand and round the Guianian highlands into the Amazon basin on the other. Specimens from north of Costa Rica and south or west of Obidos are a matt non-iridescent black.

The iridescence of the northeastern continental forms is slight and somewhat ephemeral, for it is not visible in old or rubbed specimens. It might be possible to quantify the blueness by measuring the spacing of the striae, but this was beyond the capabilities of the equipment available. From the homogeneity of the iridescence on each wing it does seem that differences in blueness are due to differences in the structure of the individual scales rather than in the relative abundance of scales exhibiting iridescence. In all the specimens studied, the degree of blueness is greatest over the proximal half of both fore and hind wings and decreases distally, as in *Heliconius cydno, wallacei, sapho, sarae* and the other species that exhibit brilliant iridescence.

All the specimens known from the valley of the Cauca river in Colombia, that is between the western and central cordilleras, exhibit a bright iridescence which has a greenish hue unknown from other localities (*chestertoni* Hewitson 1872).

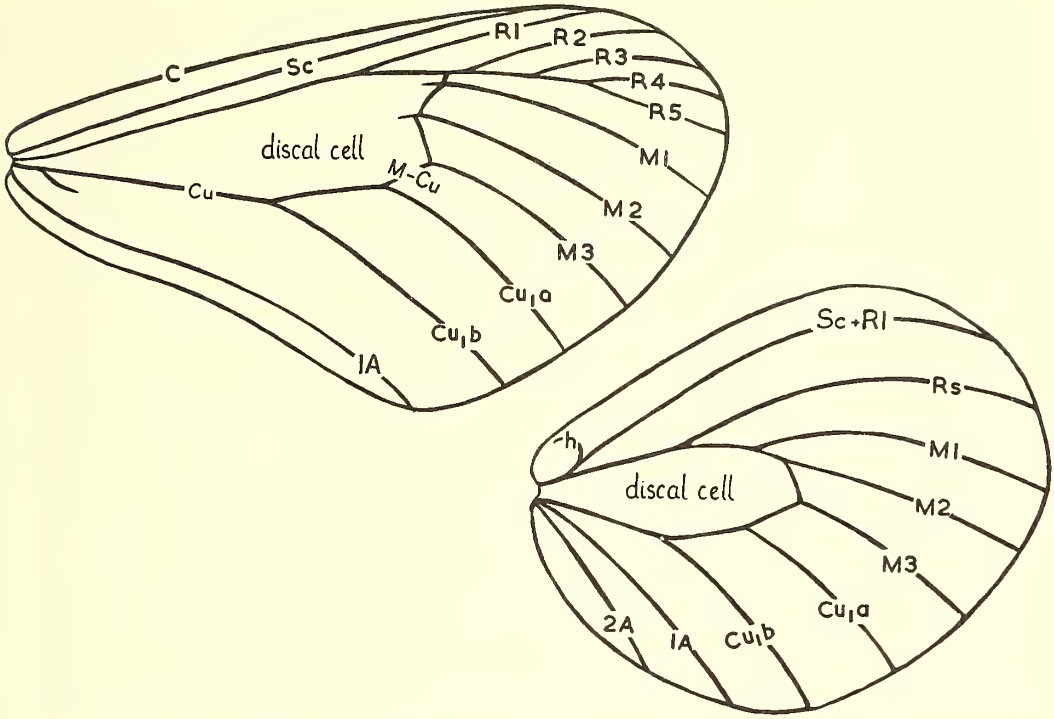
In northern Colombia, where the rate of change of blueness is greatest, there is considerable individual and local variation about a mean which is intermediate between the brilliant blue of the west coast and the dull blue of the northeastern continent.

In central British Guiana there are localities near the neighboring Essequibo and Demerara rivers where the iridescence is far in excess of the norm for that region (*magnificus* Riffarth 1900). The accuracy of the label data is confirmed by other characters.

FOREWING BAND (Pl. I, fig. 2; Pl. II, fig. 1; Text-figs. 1 & 2 A-N). The only localities where forewing band is known to be absent are as a rare polymorph in the Guianas (*oberthüri* Riffarth 1903), and as a monomorphic form in the valley of the Cauca river in Colombia (*chestertoni).*

In almost all cases the bands are grossly similar on both surfaces of the wing but the red bands are usually pale pink ventrally. As the coincidence of the bands on the two surfaces is not always exact, the descriptions and comparisions are based on dorsal characters only. Text-fig. 1 shows the conventional nomenclature of lepidopteran venation which will be used to facilitate description.

In Ecuador west of the Andes the red band is narrow along the length of the wing, and short across the width (*cyrbius,* Text-fig. 2A; Pl. I, fig. 2). There is only minor variation in band size, the narrowest of which was named *bellus* by Riffarth in 1907. Ecuadorian specimens are known (*diformatus* Riffarth 1900) in which the band is as broad as the narrower forms from western Colombia, but unfortunately the exact localities are unreliable.



TEXT-FIG. 1. Dorsal view of right fore and hindwings of *Heliconius,* illustrating venational nomenclature. **C**—costa; **Sc**—subcosta; **R1-R5**—first to fifth branch of radius; **Rs**—radial sector; **M1-M3**—first, second and third branches of the media; **Cu**—cubitus; **Cu1a** and **Cu1b**—branches of first cubitus; **1A** and **2A**—first and second anal veins; **H**—humeral branch of subcosta; **M-Cu**—medio-cubitus crossvein.

In Colombia west of the western cordilleras the band is very broad and long *(venus* Staudinger 1882, Text-fig. 2B), the increase in the width of the band taking place almost entirely in a distal direction, so the mid-point of the band is far beyond the apex of the discal cell. The boundary between *venus* and the narrow-banded *cyrbius* to the south is not revealed by the scanty locality records, and there is no obvious feature that could act as an obstruction to the free distribution of the two populations.

In the Cauca valley between the western and central cordilleras in Colombia, the only known form of *erato* is without a forewing band *(chestertoni),* but specimens are known from unspecified localities in Colombia in which there is a narrow short forewing band, like that of *cyrbius* or *bellus,* on the greenish-blue ground color known only in *chestertoni.* These specimens, named *molinus* by Grose Smith in 1898, may all be from the narrow northern open end of the Cauca valley where non-banded *chestertoni* would meet the medium-width long red-banded forms of northern Colombia. It will be shown later that the band of *cyrbius* and probably that from northern Colombia are under multifactorial control, so the intermediate character of the *molinus* band would be the expected result of such a hybrid. Other characters are also consistent with the intra-specific hybridic nature of *molinus.*

In northern Colombia, and in the Magdalena valley between the central and eastern cordilleras, the band is of similar length to that of western Colombia but is narrower and more symmetrically disposed about the apex of the discal cell *(colombinus* Staudinger 1896, *antigonus* Riffarth 1900, *guaricus* Reakirt 1868, Text-fig. 2C). Local variation is inconspicuous and is principally in the development of the emargination of the proximal boundary towards the junction of M-Cu and Cu 1 a. Intermediates between the western and northern Colombian bands are rare in museum collections and the only described form which has the anticipated appearance is *juno* Riffarth 1900 from Panama. The northern Colombian band also occurs in Panama, but beyond northern Panama the band becomes more frequently like the typically Mexican shape *(petiveranus* Doubleday 1847, *demophoon* Ménétriés 1857, Text fig. 2D), though intermediate bands like that of *tristis* Riffarth 1900 occur rarely as far north as Nicaragua.

East of the eastern cordilleras through the

Orinoco basin to the northeast of the Guianian highlands and into the Guianas, the band is red, long, broad and centered symmetrically over the apex of the discal cell (Text-fig. 2E). In the Guianas and along the lower Amazon to the southwest as far as Obidos, this band occurs polymorphically, but at low frequencies, with bands of other colors and shapes, but to the northeast it is monomorphic and it is quite typical in Trinidad. The name *hydarus* Hewitson 1867 has been applied to Trinidadian material, but not only is the band of the type-specimen of *hydarus* of atypical shape but there are a pair of unique red spots over the apices of the discal cells of the hindwings unknown in any specimens from Trinidad. Furthermore, the type locality of *hydarus* is Colombia, the majority of which is beyond the zone for which the Trinidad band-type is characteristic. Named forms with a band that is typical of the northeastern area of South America are *adanus* Seitz 1913, *vitellinus* Stichel 1919, both from Trinidad, and *dryope* Riffarth 1900, from the Guianas and lower Amazon.

The high degree of polymorphism in the Guianas is best understood after the monomorphic populations have been described.

Along the coastal strip of Brazil that borders the eastern side of the highlands, through Paraná, Rio Grande do sul, Paraguay and extreme northeastern Bolivia, the red forewing band is a characteristic crescentic shape with a distally bidentate margin *(phyllis* (Fabricius 1775*),* Text-fig. 2F). The continuity of the red-banded forms from the polymorphism of the Guianas to the monomorphic *phyllis* zone is uncertain and is discussed later.

Along the eastern slopes of the Andes there are different forms of forewing band localized in valley systems and restricted to relatively narrow altitudinal limits. The dearth of accurate data from the Peruvian Andes makes the interpretation very unreliable, but the situation is probably very similar to that in eastern Ecuador where the author has personal experience.

The central region of South America, which is drained by the Amazon and its major tributaries, has exclusively yellow-banded forms of *erato,* but where these meet the red-banded forms, which have already been noticed in the Guianas, eastern Bolivia and east-central Colombia, there are polymorphic zones exhibiting red, yellow and red, and yellow bands. Hence, the large number of named forms. Along the Andes the upper altitudes have exclusively red or red and white banded forms, so the yellow bands of the central area are entirely surrounded by red, (Plate II, fig. 1).

The vague locality data on museum specimens from eastern Ecuador would lead one to believe it was an area of considerable polymorphism, but this is not the case. In Ecuador at Pastaza (Shell Mera) the only form present is *notabilis* Salvin & Godman 1868, which has a matt black ground color and a double band which is white with a narrow red peripheral border and with red in the posterior half of the proximal band (Text-fig. 2G). The amount of red varies considerably, as does the degree of separation of the two bands, for some examples are all white, others all red, but there is a tendency for the higher-altitude specimens to have the greater proportion of red on the bands, though in almost all specimens there is a variably small number of yellow scales intermingled with the peripheral red. One example was collected from a total of 52 from Pastaza in which the distal half of the outer band was missing, leaving the outer band as a narrow segment, a condition also known from museum collections and laboratory-reared specimens, and later referred to as "truncate."

Though *notabilis* seems to form the upper limit of the species in Ecuador at 1,150 meters, it descends to about 900 meters, at which level there are infrequent irregularities in the band shape with a tendency for them to fuse *(rosaceus* Riffarth 1907), and at 850 meters typical *notabilis* has been replaced by variable double white- and double yellow-banded forms, which may or may not carry dennis-ray patterns (see below). There was no opportunity to collect at altitudes between this and the 650 meter level, at which the band is monomorphically single and pure yellow, and there are few recognizably intermediate specimens in the museums visited (*ochraceus* Riffarth 1907). In the valleys of the Santiago river near Macas the outer band occurs in the total absence of the inner band (*etylus* Salvin 1871, Text-fig. 2M).

In the valleys of the upper tributaries of the River Huallaga, in northeastern Peru, there is a stable single-banded form, *favorinus* Hopffer 1874 (Text-fig. 2H) and *eratophilus* Joicey & Kaye 1917, the band shape of which is not unlike that from Trinidad, but it is yellowish-buff beneath. The only reason why forms intermediate between *favorinus* and the yellow-banded forms to the east are unknown seems the extreme inaccessibility of the area. In the valleys of the Chanchamayo and Perené rivers, which are tributaries in the complex system of the River Ucayali, there is a double-banded form (*microcleus* Kaye 1907, Text-fig. 2I), which is similar to *notabilis* but has much larger all-red bands. It probably occupies an ecological zone comparable to that occupied by *notabilis* in Ecuador.

Contrary to some data labels it is doubtful if *microcleus* ever exceeds 1,200 meters.

From a number of localities along the valleys of the terminal tributaries of the Beni and Madre de Dios rivers, like the Rivers Inambari and Songo, specimens of *erato* are known in which the red forewing band is a very broad long or short rectangle with a more or less prominent black crescent at the apex of the discal cell (*amphitrite* Riffarth 1901, Text-fig. 2J) and *unipunctatus* Joicey & Kaye 1917. As with *favorinus* the bands of specimens from the more upstream localities are yellow underneath.

The yellow-banded forms from the Amazon basin have two basic patterns from which all the remaining forms can be achieved by differential reduction and combination. The dominant pattern in the eastern continent is that of *H. erato erato* (Linnaeus 1758) (Text-fig. 2K) in which the band is divided across the apex of the discal cell and distally broken into spots. It is the major component of the polymorphic complex in the Guianas and lower Amazon, and may persist monomorphically in the coastal region to the east. The data on this point are unreliable, for the locality "Para" may mean anywhere in the province of Para and not necessarily near the city of Belem. Further doubt is cast on the yellow form reaching the coast monomorphically by the presence of yellow and red combinations to the southeast where red *phyllis* is definitely known from Marenham, together with combinations of *phyllis* and northern long red band. However, against this is the fact that no band form other than broken yellow is known from Belem or any locality between Bragance and the northern side of the Amazon estuary.

The principal sites of variation in this broken band are the posterior, anterior, proximal and distal quarters, each or combinations of which may be reduced to varying degrees. These variations may be combined with compaction of the spots and variation in the proportion of red to yellow, brought about by admixture of characters from other band forms. The extensive variation in the Guianas has led to a large number of named forms which really only define steps in a continuously varying population. The phenotypic characters of those forms represented in the British Museum, together with a few others whose descriptions are adequate, are classified in Table I.

As one proceeds westward along the Amazon the frequency of complete broken-band forms decreases, for there is regular increase in the degree and frequency of reduction of the proximal half of the band. By Teffé almost all specimens have only that part of the broken band that lies outside the discal cell represented, though some still retain a vestige in the antero-distal angle (*lativittus* Butler 1877, *divus* Stichel 1906). Above the contour passing through Iquitos the band is completely outside the discal cell and is of shorter but more variable length (*estrellus* Bates 1862, *emmus* Riffarth 1901, Text-fig. 2L). As mentioned above, undoubted intermediates between the *emmus* band and the double *notabilis* band of higher altitudes are uncommon.

The second elemental yellow band shape from the Amazon basin is more compact, short in length and centered over the apex of the discal cell. The typical form, *venustus* Salvin 1871 (Text-fig. 2N), is characteristic of the valleys of the tributaries of the River Madeira in west-central Brazil but this "compact" band invades the northern area dominated by "broken" band, and intermediates such as *udalricus* (Cramer 1780) from the Guianas and the lower and middle Amazon may be due to the combination of "broken" and "compact" band characters.

In the Guianas the variety is even more complex because of the addition of long broad band from the north and perhaps some characters unknown elsewhere. The super-broad band of *magnificus* from British Guiana, which is superficially like that of *amphitrite* (Text-fig. 2J), is relatively constant and seems associated with the more brilliant iridescence than that of neighboring forms, through there is no genetical evidence for linkage.

In the shallow valleys of the rivers Mamoré, Guaporé and Rio Grande, which drain central Bolivia, the red-banded forms from the eastern Andes and Paraguay meet the compact yellow bands of the lowlands. The result is a polymorphism which approaches that of the Guianas in its complexity. The combinations of *venustus* yellow-compact band, southern *phyllis* crescentic-red band and the super-broad-red band of *amphitrite* produce a host of intermediates, the named forms of which are phenotypically classified in Table I.

The only other locality where the absence of an altitudinal barrier allows the central region yellow-banded forms to meet peripheral red-banded forms over a broad area is in south-central Colombia between the eastern cordilleras and the llanos of Venezuela, though there seems also to be a leak through the southern end of the Magdalena valley. The number of specimens from this area is small, presumably because of inaccessibility, but they all show combinations of characters as in the Guianas and Bolivia. They have been included in the phenotypic classification in Table I.

FOREWING FLECK. The yellow or white patches that may occur proximally on the forewing band of *melpomene* in western Ecuador are unknown in *erato*.

DENNIS (Pl. I, fig. 1; Pl. II, fig. 2). The name "dennis" was given by Turner & Crane (1962) to the element of pattern in which the approximate basal third of the forewing is red (Pl. I, fig. 1). The amount of red is variable, particularly in the Guianas, but except in southwestern Brazil it is prominent on both wing surfaces, though in no case does it extend over the posterior part of the forewing that normally covers the anterior part of the hindwing. Together with the red forewing base there may be a trace of red at the extreme base of the hind wing, but not so as to form a longitudinal bar as in *melpomene* (Pl. I, fig. 3). The geographical area over which dennis occurs monomorphically extends westward from Obidos to the slopes of the Andes at an altitude in Ecuador of about 850 meters, and southward around the plateau of Matto Grosso to Bolivia and Peru up to an altitude of about 300 meters (Pl. II, fig. 2). To the northeast of Obidos dennis and non-dennis occur in the same polymorphic population as far as British Guiana, but beyond that dennis is unrecorded. A similar polymorphism is found in central Bolivia, along a narrow altitudinal zone in the eastern Andes and in southern Colombia where dennis again makes an interface with non-dennis. In specimens exhibiting dennis and double band, dennis is separated from the proximal boundary of the inner band by a narrow line of ground color (as in Pl. I, fig. 1), but when it occurs with single band it may be a little more extensive distally and less precisely defined. The presence or absence of dennis has greatly multiplied the number of named forms, and these are included in Table I.

RAY (Pl. I, fig. 5; Pl. II, fig. 2). Usually occurring with dennis, but rarely on its own *(anaitis* Riffarth 1907), ray (Turner & Crane, 1962) is a series of radiating lines emanating from a red discal bar on the upper and lower surfaces of the hind wing (Pl. I, fig. 5), though most frequently the upper surface rays are more prominent. Ray is composed essentially of a red center to the discal cell and red lines in the open cells behind each of the radiating veins Sc+R1, Rs, M1, M2, M3, Cula, Culb, 1A and 2A, but the rays behind Sc+R1, and Rs are not expressed in males, as androconia are borne on the dorsal surface of that area. The red on the discal cell, and/or that behind Culb, may divide to reveal a central streak of ground color. The width and intensity of the rays in the middle and lower Amazon is greater than elsewhere *(amazonus* Staudinger 1896 and other forms) though there is variation in all localities. The gross distribution of ray coincides exactly with that of dennis (Pl. II, fig. 2), but whereas the occurrence of dennis without ray is common in the Guianas, it is much less common in the other polymorphic zones. These combinations have contributed to the large number of named forms tabulated in Table I.

HINDWING YELLOW BARS (Pl. I, figs. 4 and 7; Pl. II, fig. 2). In western Ecuador and western Colombia there is a longitudinal yellow bar centered over the posterior margin of the discal cell on the ventral surface of the hindwing (Pl. I, fig. 7). The heterozygous condition of this bar is a similarly located but barely perceptible differentiation of the brown ground color (see genetics section), and is known in the wild from the Cauca valley (*nocturnus* Riffarth 1900), from Panama (*juno* and *tristis*), northern (and rarely eastern) Colombia (*guaricus*) and Bolivia (*clelius* Neustetter 1927, *phyllidis* Grose Smith 1892). However, the true distribution of ventral yellow bar is masked, for dorsal yellow bar, which is a coincident yellow bar on the dorsal surface (Pl. I, fig. 4), seems also to express itself ventrally. No instances are known of dorsal bar in the absence of ventral bar. Dorsal bar extends monomorphically from northern Panama to Mexico, (*demophoon* and *petiveranus*), along the Cauca valley in northern Colombia (*chestertoni*), along the upper tributaries of the Huallaga river in northern Peru (*favorinus*) and around the coastal strip of Brazil, through Paraguay to eastern Bolivia *(phyllis)*. The bar occurs polymorphically in the Magdalena valley, northern Colombia and Panama, and with a high incidence of apparent heterozygosity in east-central Bolivia. These localities are all areas on the interface between bar and non-bar forms. The nature of the heterozygotic expression of dorsal bar is without experimental evidence, but in Bolivia, where the variation is most diverse, the phenotypic expression of bar may take one of the following four forms: a complete dense bar on both surfaces, sparsely scattered yellow scales on both bar surfaces, restricted dense portions of bar on both surfaces, and localized dense portions of bar on the dorsal surface only. It will be shown in the next section that ventral bar is controlled by a single pair of allelomorphs but as it is possible that there is interaction between the expression of dorsal and ventral yellow bar, and there may be more than one controlling gene for dorsal yellow bar, further analysis has not been attempted. Additional evidence is available in the combinations of bar with ray in Bolivia. There, bar may overprint the rays, be overprinted by the rays, be under the rays but overprint the normally black veins, or be masked by both rays and veins. It should be noted that whereas the evidence from Bolivia suggests that the heterozygous condition of dorsal bar should be recognizable, very few specimens with an intermediate condition between dorsal bar and non-bar are known from northern Colombia and Panama. The few possibles that are known (e.g., *nocturnus*) show a very sparse scattering of yellow scales over the dorsal bar position, which is a condition that has appeared in the laboratory F_2 progeny of *cyrbius* and *adanus* crosses. As *cyrbius* has only ventral yellow bar, even this character may not be attributable with certainty to dorsal yellow bar.

There is considerable regional variation in the shape of bars. In western Ecuador the ventral bar of *cyrbius* is variable in intensity, but always long, narrow and with a vague periphery. In western Colombia the ventral bar of *venus* is about twice as wide as that of *cyrbius* and uniformly dense yellow with a precise boundary. The dorsal and ventral bar of northern Colombia, Panama and Costa Rica is shorter but broader with a ragged periphery, whereas from Guatemala to Mexico it is similar to the narrow bar of western Ecuador but uniformly intense and with a precise boundary. The change-over takes place about Nicaragua, as does the change in iridescence and band shape, though in the transitional localities both types of bar and intermediates may be found. The bar of the Huallaga river system has a very precise boundary but is otherwise similar to the short broad bar of northern Colombia; there is also a similar absence of recognizable heterozygotes, but this may be due to the inaccessability of the locality and the relatively small number of specimens known. In central Bolivia and south Brazil the narrow long bar again has the margins more precisely defined and with a smoother surface texture like that from Central America north of Costa Rica. Though the regional differences in bar shape and texture could be due to geographically distinct modifying genes, the apparent difference in the heterozygous behavior suggests that more than one major gene is involved. There are as yet no experimental data available.

HINDWING WHITE MARGIN AND FRINGE (Pl. I, fig. 4). In western Ecuador the terminal scales at the periphery of both fore and hindwings of *cyrbius* are totally white, and on both the upper and lower surfaces of the hindwing the white invades the membrane from a pair of centers between the terminations of each pair of veins. The proximal boundary of the paired white hindwing patches are asymmetrically convex. Within the Ecuadorian populations there is little variation but near the boundary with *venus* in Colombia the width and density of the margin is variable. There may be some yellow scales intermingled with the white, but the yellowing always proceeds from the proximal margin.

In western Colombia north of a line approximating to the River Patia the white margin is reduced abruptly to a complete fringe of white scales with no encroachment onto the membrane *(venus).*

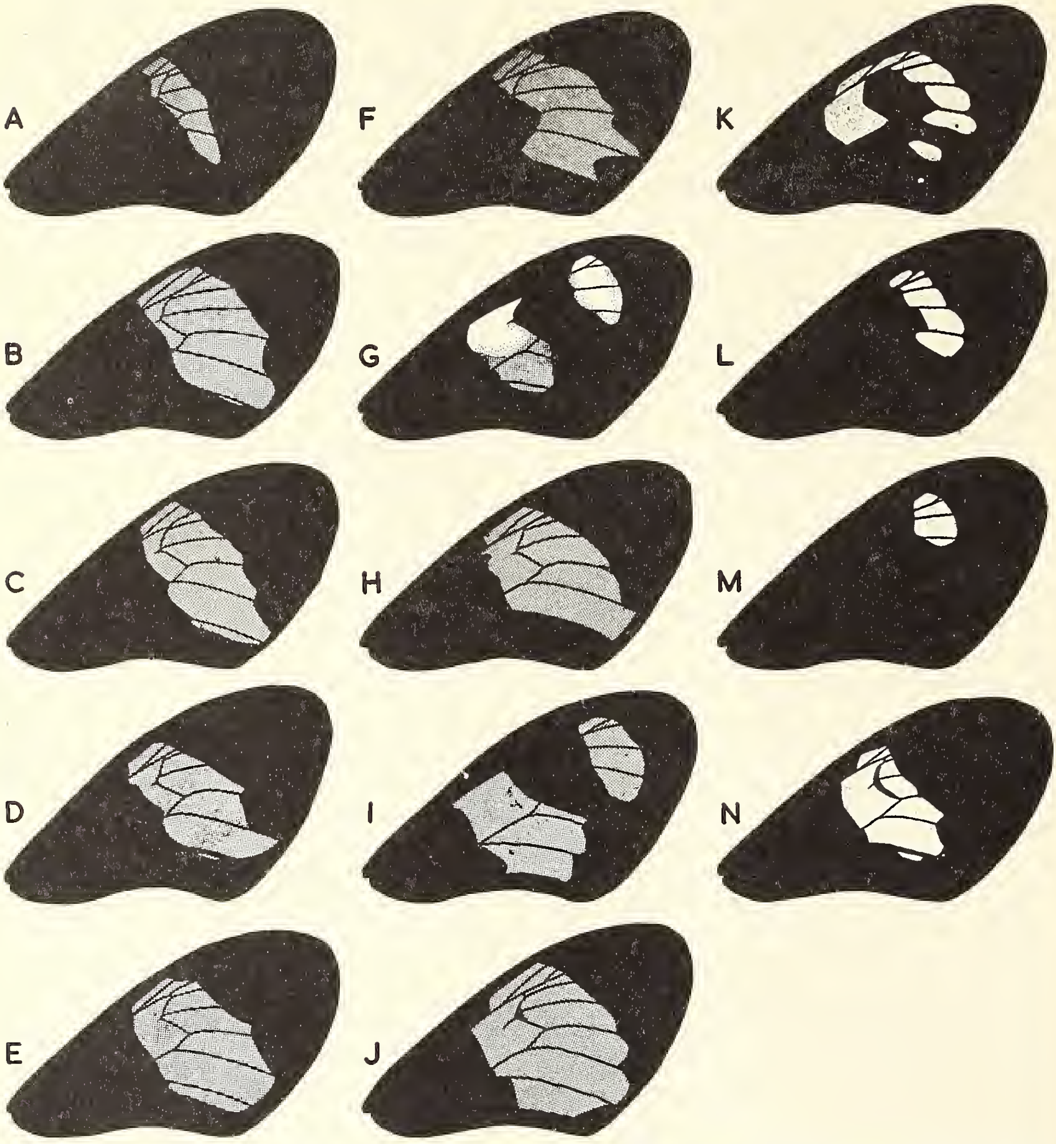
In the Cauca valley and elsewhere over the whole range of *erato* there are no white scales on the margin of either fore or hindwing, except for the rare occurrence in west-central Bolivia of forms in which there are a pair of small submarginal intervenal white spots on the posterior border of the hindwing, and associated with which are double radiating lines of white scales as in *Heliconius wallacei, doris, aoede, xanthocles* and several other species.

FOREWING YELLOW LINE (Pl. I, figs. 1 and 2). This character is a narrow yellow line on both surfaces of the forewing which lies slightly obliquely over Cu1 and which terminates just distal to the fork of Cu1 into Cu1a and Cu1b. In *erato* it occurs only in coastal and southern Brazil, Paraguay and eastern Bolivia (*phyllis*), but it is also a feature of *Heliconius wallacei, sarae, antiochus, doris* and several other species.

The yellow spot at the dorsal base of the forewing, which occurs as a rarity in the Guianas but becomes very frequent among Amazonian populations, is coincident with the proximal origin of the forewing line. In the line/non-line zone of eastern Bolivia there is considerable polymorphism as well as variation in the intensity of expression of line.

FOREWING COSTAL SPOT. This is a small rectangle of red at the ventral base of the forewing, lying between the costal and subcostal veins. It is a feature of *erato* everywhere except in the Cauca valley where it is absent, though in western Ecuador and at the higher levels in the eastern Ecuadorian and Peruvian Andes it is variable. In 160 museum specimens from the Quevado district of western Ecuador, 96 were without any evidence of a costal spot and in the remainder there was considerable variation in its size. In forms exhibiting dennis the costal spot is usually masked, though it may sometimes be detected as a more intense red.

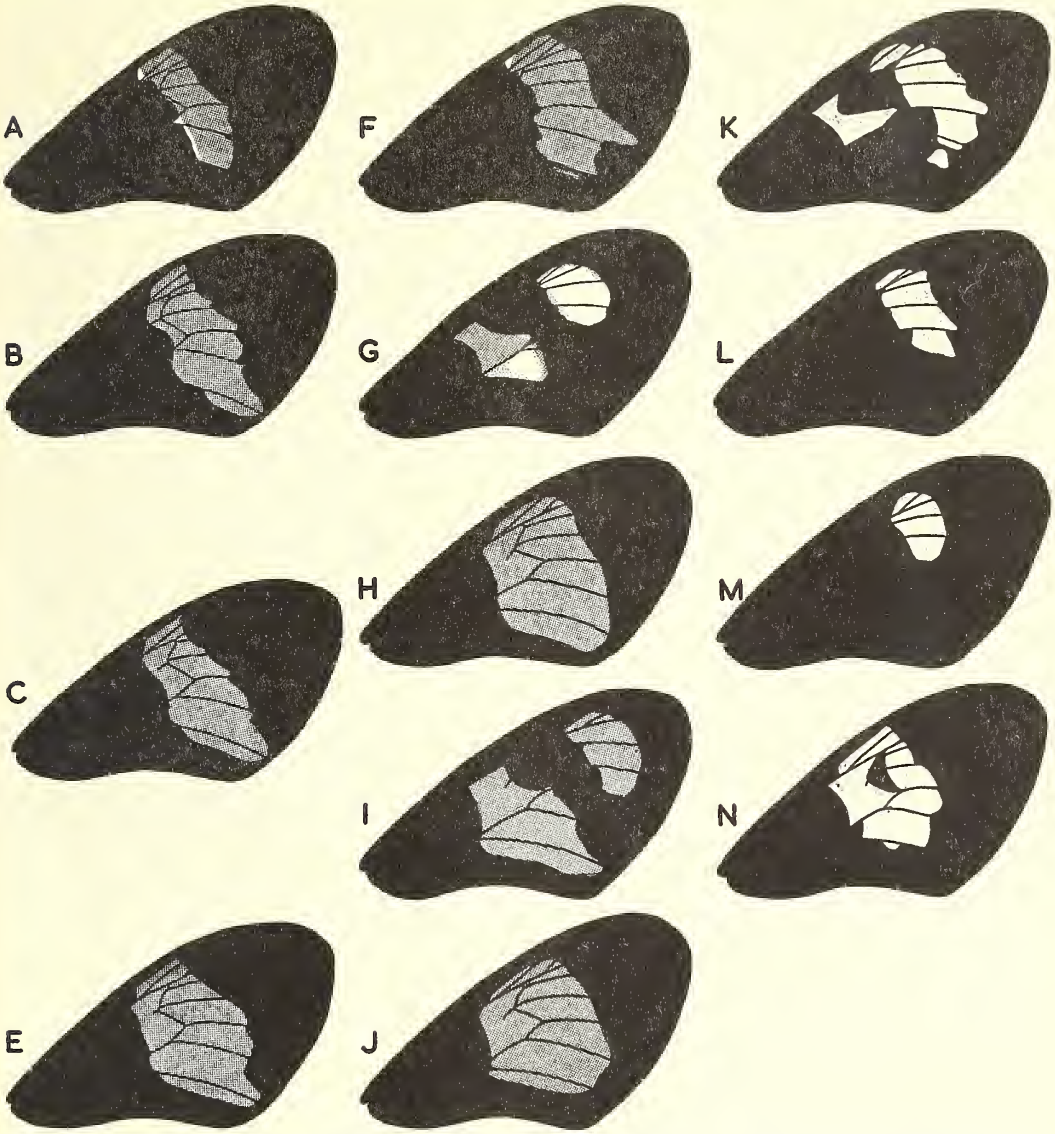
HINDWING COSTAL STREAK (Pl. I, fig. 7) is a yellow border along the leading edge of the ventral surface of the hindwing, which is most broad inside the recurrent branch of the subcostal vein and which tapers off midway along the length of the wing. In forms exhibiting ray, the yellow



TEXT-FIG. 2. Right dorsal forewing bands of *Heliconius erato*. The shaded areas on figures A-J are red and those of K-N are yellow. **A**—*cyrbius*, western Ecuador; **B**—*venus*, western Colombia; **C**—*colombinus*, northern Colombia; **D**—*petiveranus*, northern Central America; **E**—*adanus*, northeastern South America and Trinidad; **F**—*phyllis*, eastern and southern Brazil; **G**—*notabilis*, 800-1,100 meters in eastern Ecuador; **H**—*favorinus*, upper River Huallaga, northeast Peru; **I**—*microcleus*, upper River Ucayali, central-eastern Peru; **J**—*amphitrite*, upper Beni and Madre de Dios rivers, southeastern Peru; **K**—*erato*, the broken band of the Guianas and middle and lower-middle Amazons; **L**—*emmus*, upper and middle-upper western Amazons; **M**—*etylus*, upper Santiago river system in southeastern Ecuador; **N**—*venustus*, the compact band of southern middle Amazons. All approximately 1½ times natural size.

may be reduced to a few basal scales, but some yellow can always be identified. Though locally variable in extent, the only locality from which the yellow costal streak is absent is in the Cauca valley (*chestertoni*).

HINDWING BASAL SPOTS (Pl. I, fig. 7). These are a cluster of up to four red spots at the ventral base of the hindwing. In western Ecuador they are very small though distinct, but in western Colombia the number varies from none to four. In the Cauca valley *chestertoni* may have none or one, and the intermediate *molinus* two to four. Elsewhere four is the rule, and even where they are masked by ray, their presence can usually



Text-fig. 3. Right dorsal forewing bands of *Heliconius melpomene*. The shaded areas on figures A-C and E-J are red and those of K-N are yellow. A—*cytherus*, western Ecuador; B—*vulcanus*, western Colombia; C—*rosinus*, northern Colombia and Central America; E—*flagrans*, northeastern South America and Trinidad; F—*besckei*, southeastern Brazil; G—*plesseni*, 800-1,100 meters in eastern Ecuador; H—*amaryllis*, upper River Huallaga, northeast Peru; I—*xenocleus*, upper River Ucayali, central-eastern Peru; J—*euryades*, upper Beni and Madre de Dios rivers, southeastern Peru; K—*thelxiope*, the broken band of the Guianas and middle and lower-middle Amazons; L—*elevatus*, upper and middle-upper western Amazons; M—*ecuadorensis*, upper Santiago river system in southeastern Ecuador; N—*vicinus*, the compact band of southern middle Amazons. All approximately 1½ times natural size.

be detected by the more intense red at the spot locations.

Hindwing Apical Cream Rectangles (Pl. I, fig. 7). Specimens from south and southeastern Brazil typically have two or three cream rectangles set subapically at a tangent to the periphery of the ventral surface of the hindwing, just beyond the distal extremity of the ventral yellow bar. In central Bolivia this character becomes variable in presence as the population merges with the Amazonian one in which this character is absent. Specimens have been seen in which the site of the rectangles is perceptible as a slight differentiation of the ground color; this may be the heterozygous condition.

HINDWING VENTRAL RED RAYLETS (Pl. I, fig. 7). Specimens from south and southeastern Brazil may have a line of three to six red triangular raylets posterior to the ventral yellow bar, one between each of the hindwing veins (*artifex*). The proximal pair may be double.

BODY COLOR. Though the body is mainly black, there are light markings on the head, thorax and abdomen, which are predominantly yellow everywhere except in specimens from western Ecuador, western Colombia and the Cauca valley, in which cases they are white. Outside these localities the yellow head markings bear a variable quantity of white scales which are most abundant on the lateral occiput, the frons and the antennal bases. The partial yellow color of the palps serves to distinguish *erato*, and usually *melpomene*, from the very similar sympatric species *aoede, xanthocles, egerius* and *burneyi*, in all of which the palps are pure white.

Heliconius melpomene (Linnaeus 1702)

GROUND COLOR (Pl. I, figs. 1, 3, 6 and 8). The situation is similar to that pertaining in *erato* except that in *melpomene* the blue iridescence is always a little less intense than that of sympatric forms of *erato*. No super-brilliant specimens like the Guianese *erato magnificus* are known.

FOREWING BAND (Pl. II, fig. 1; Text-figs. 3A–N). In western Ecuador the band of *cytherus* Hewitson 1869 is similar in color, shape, location and relative size to that of the sympatric *cyrbius* (cf. Text-fig. 2A & 3A), the only conspicuous feature distinguishing the *melpomene* band being the whitening of the proximal margin which is here termed "forewing fleck."

In western Colombia the band of *vulcanus* Butler 1865 is broad and long and only just fails to meet the posterior margin of the wing (Text-fig. 3B). Distally there is emargination of the band along the veins, particularly along Cu1a, so the band is slightly narrower than that of the sympatric *venus* and more symmetrically disposed about the apex of the discal cell. There are insufficient localities to determine the geographical boundary with *cytherus*.

In northern Colombia, excluding the Cauca valley where *melpomene* is unknown, and northward into Central America, the forewing band of *rosinus* Boisduval 1870 and *euryas* Bates 1862 is of medium width but long and similar to the sympatric forms of *erato* (cf. Text-figs. 2C & 3C). Unlike *erato* there is no differentiation of a characteristic northern Central American band shape, for *melpomene* is not known to occur north of Nicaragua.

To the east of the eastern cordilleras in Colombia, the band of *melpomene* (Linnaeus 1702) and *flagrans* Stichel 1919 is long, broad and red but though similar in shape and distribution to that of the sympatric *erato* (cf. Text-figs. 2E and 3E), it is much less common in the polymorphic complex of the Guianas and lower Amazon. It is again most convenient to leave the consideration of the polymorphic areas until last.

The southern forms comparable in appearance and distribution with *erato phyllis* are *besckei* Ménétriés 1857 and *nannus* Stichel 1899 (cf. Text-figs. 2F & 3F). Though no *melpomene* are known from the east coast of Brazil, even *erato* records are very few and it is possible that *melpomene* has been overlooked. One would expect to find the *besckei* band in this area.

The zonation in eastern Ecuador parallels that of *erato* exactly, with *plesseni* Riffarth 1907 flying in the *notabilis* zone (cf. Text-figs. 2G & 3G), and with many varieties occurring along the interface between the stratum containing the double red and white band non dennis-ray form and that containing the single yellow band dennis-ray form; some of those named are included in Table I.

In the terminal valleys of the Huallaga river system the forewing band of *amaryllis* Felder 1862 is long, broad, single and red and is rather similar to that from the northeastern part of the continent, but it also approximates quite closely to that of the sympatric *erato favorinus* (cf. Text-figs. 2H & 3H). Specimens are known from Chazuta (300 meters) in which there are proximal yellow scales on the red band, so this is probably near the downstream boundary with the all-yellow band forms. *Amaryllis* extends along the Huallaga river at least as far as Tingo Maria (750 meters), where it almost unites with *xenocleus*.

Xenocleus Hewitson 1852 is the characteristic double red-banded form of the upper Perené river where it coincides with the very similar *erato microcleus* (cf. Text-figs. 2I & 3I).

The large red band of *erato amphitrite* in the upper Madre de Dios river is matched by that of the sympatric *euryades* Riffarth 1900 (cf. Text-figs. 2J & 3J).

As in *erato*, the Amazon basin is occupied by yellow-banded forms, but though the total effect is similar, their constitution is more complex. One of the basic patterns in the middle Amazon is a "broken" band typified by *thelxiope* (Hübner 1806) (Text-fig. 3K) and, as in *erato*, there is reduction of the proximal and posterior portions of the band as one travels in a westward direction. This trend produces such forms as *agalope* Felder 1862 and *elevatus* Nöldner 1901 (Text-fig. 3L) before reaching the very reduced band of *ecuadorensis* Neustetter 1908 (Text-fig. 3M). This oval band occurs in the valley of the upper Napo at Archidona as well as in the upper Santiago river district, but it seems unlikely that the *ecuadorensis* band is homologous with that of *erato etylus*, for its more proximal location suggests that it is a remnant of the antero-distal portion of the broken band rather than a distinct character. The comparable band in *erato etylus* is of more restricted geographical distribution and is probably homologous with the outer band of the double-banded *notabilis*.

There is a compact band type exemplified by *vicinus* Ménétriés 1875 (Text-fig. 3N) which is most frequent in the southwestern Amazons, but which does invade the lower and middle Amazon area at lower frequency, as in *erato*.

The extreme complexity of band in *melpomene* is principally due to the occurrence, at high frequency in the Guianas and at decreasing frequency to the south and west, of what is here called the Z-band (not figured because of its variability). Sheppard (1963) founded it in his material, and in his breeding program the homozygous condition appeared as a broken yellow band with a distal narrow long band of red. The heterozygous condition, in combination with Trinidad long broad red band (*adanus*), had the yellow reduced proximally, but it seems that there may be a series of allelomorphs at this locus, so more breeding data are required. The distinguishing feature of the band is the sharp interface between the red and yellow which lies along a transverse line which is deflected proximally in cell Cu1a through about fifty degrees. A similar interface also occurs in *melpomene heurippus,* which is not considered a distinct species, and in the wild hybrids *rubellius, wernickei* and *emilius.* The red spot at the posterior forewing margin of *timaretus* and its polymorphs is the posterior vestige of this band. In the intraspecific hybridization zone of central Bolivia the red and yellow band combinations only rarely have the Z-band type of interface, it being much more common for the red and yellow scales to intermingle over a broad area; in these individuals the Z-band character is considered to be absent.

In Para, the Z-band character, with broken yellow band, extends as far as Bragance on the coast so it is unlikely that the population of either *melpomene* or *erato* is strictly monomorphic in this area.

The taxonomic status of *tumatumari* Kaye 1906 and *bari* Oberthür 1902 has not been confirmed, but if they are *melpomene*, as seems most likely, they are the only forms in which there is a subapical band beyond the principal broken yellow band. This is a character of wide occurrence in other species.

Forewing Fleck (Text-fig. 3A). In western Ecuador there may be up to three white or partly yellow flecks associated with the proximal margin of the forewing band. It is probably better to regard these flecks as lightening of the band rather than as discreet items. The flecks comprise a conspicuous light rectangle over the origin of R1, a less conspicuous triangle in the angle between the M-Cu cross-vein and Cu1a, and a vague area which may be reduced to a few light scales along the posterior proximal margin of the band. The expression of the white is variable and any of the flecks may be reduced to a few scales, or they may coalesce and form an irregular transverse white strip along the proximal border of the band. This variation in the extent and color of the flecks has given rise to the naming of several forms. North of the River Patia, in western Colombia, fleck is less common than in Ecuador, in northern Colombia it is a rarity, and elsewhere the only locality in which light fleck is known is southeastern Brazil where the yellow spot over the origin of R1 is a constant feature of *besckei*.

Dennis (Pl. I, figs. 1 and 3; Pl. II, fig. 2). Though dennis on the forewing is similar in *melpomene* and *erato,* the considerable difference between the two on the hindwing is illustrated on Pl. I, figs. 3 & 4, where it is shown that in *erato* dennis has little or no expression on the hindwing whereas in *melpomene* there is a narrow red longitudinal bar which is always expressed in combination with the red base to the forewing. The dennis markings appear on both surfaces of the wing, though in southwestern Brazil the expression of dennis on the ventral surface is weak, and they may occur with or without ray (see below). The geographical distribution will be discussed under ray, for most commonly these characters are associated together.

The narrow posteriorly concave bar of dennis is frequently enlarged by the addition of a biconvex red bar which in shape is not unlike the yellow dorsal bar discussed below. The presence or absence of the biconvex bar accounts for the discontinuous variation in the breadth of the gap between the bar and the anterior margin of the rays when they are present. The only example known in which the broad red bar occurs in the absence of dennis is *amandoides* Neustetter 1926, though in *margaritus* Riffarth 1900 the yellow dorsal bar has red scales intermingled. Both are from Bolivia.

Ray (Pl. I, fig. 6; Pl. II, fig. 2). Ray in *erato* and *melpomene* differs in appearance, for whereas *erato* has the rays radiating from a red center to the discal cell, in *melpomene* the rays emanate from the bar of dennis if present, or if not, then from the position where it would be. The rays in eastern Ecuador and Peru may be proximally thickened so they resemble nails with large heads; the former is the only locality in which rays are known to occur in the absence of dennis (*virgatus* Stichel 1902). Though the inheritance of dennis and ray is distinct, ray on its own is not yet recorded from the Guianas. The distribution of dennis and ray is similar and extends as shown in Pl. II, fig. 2, over the whole of the Amazon basin. Of special interest is the limitation of dennis and ray to below 850 meters in the eastern Ecuadorian Andes, which is the altitude at which yellow band is also limited. Red forewing band and dennis-ray combine only in the peripheral polymorphic populations. The only exception to the restriction of yellow band and dennis-ray to 850 meters is the occurrence of *timaretus* above 1,250 meters in the Pastaza valley, where it occurs beyond the range of *erato*. As discussed later it is considered a relic.

HINDWING YELLOW BARS (Pl. I, fig. 8; Pl. II, fig. 2). The geographical distribution of the dorsal and ventral yellow bars in *melpomene* is comparable with that in *erato*, but the appearance of the bars differs in detail. In western Ecuador (*cytherus*), western Colombia (*vulcanus*), northern Colombia and Central America (*rosinus*), the bars are far less varied than in *erato* and are of medium width with the edges of the bars clearly defined in all localities except western Ecuador.

Just as in *erato*, the heterozygous ventral yellow bar is a faint differentiation of the ground color which is known in the wild only from the Magdalena valley, northern Colombia, Panama (*euryas*) and Bolivia. No obvious heterozygous condition of the dorsal yellow bar is known, nor is the dorsal bar known to occur in the absence of the ventral bar.

Both bars are present with a crisp boundary in *amaryllis* which coincides exactly with *erato favorinus* in the valleys of the tributaries of the Huallaga river. The bar/non-bar boundary seems to approximate to Balsapuerto (225 meters), which is near Yurimaguas in northeastern Peru.

The yellow bars of *besckei* and *nannus* coincide with those of *erato phyllis* in southern and southeastern Brazil, and in central Bolivia there is a similar degree of polymorphism at the interface with non-bar forms. Though the heterozygous (?) behavior of the bars in this polymorphic zone is directly comparable with that of *erato*, the shape of the bars is more variable, there being a particularly common form in which the bar is parallel-sided and almost rectangular (Plate I, fig. 8).

FOREWING YELLOW LINE (Pl. I, fig. 1). Though similar both in appearance and distribution to that of *erato*, it occurs also in western Ecuador (*cytherus*), where it is prominent only on the ventral surface.

FOREWING COSTAL SPOT. Morphologically similar to that of *erato*, it is also a constant feature over the whole range of *melpomene* except in western Ecuador, western Colombia and the valley of the Huallaga river, where it is of variable occurrence.

HINDWING COSTAL STREAK (Pl. I, fig. 8). Similar to that of *erato*, it is present everywhere, though when in association with ray it may be reduced to a yellow spot enclosed by the recurrent humeral branch of the subcosta. It seems somewhat variable in presence in eastern Ecuador at about 900 meters, and may be red (*ecuadorensis*) or red and yellow (*aurofasciatus* Neustetter 1928).

HINDWING BASAL SPOTS (Pl. I, fig. 8). Though similar in location to those of *erato*, the maximum number of round spots is three, for the red mark between Rs and Cu is either a small triangular fleck or absent. In western Ecuador (*cytherus*) and western Colombia (*vulcanus*) the spots are reduced to one or two and in the valley of the Huallaga river (*amaryllis*) they are frequently absent, as in the sympatric *erato favorinus*. In south Brazil the number varies between two and four, and when present the spot between Rs and Cu is more rounded as in *erato*.

HINDWING APICAL CREAM RECTANGLES (Pl. I, fig. 8). The only comparable characters in *melpomene* are the tangential cream rectangles that are continuous with the terminations of the red submarginal arcs of *besckei*.

HINDWING VENTRAL RED ARCS (Pl. I, fig. 8). An effect of red on the hindwing is obtained in the form *besckei* by a row of narrow red submarginal arcs, which lie in intervenal pairs around the posterior border of the hindwing, an effect which seems to be obtained in *erato* by the red raylets of the sympatric *phyllis* and *artifex*.

BODY COLOR. The light parts of the body are normally yellow with white at the base of the antennae and sometimes on the frons and sides of the occiput, but *cytherus* from western Ecuador and *vulcanus* from western Colombia always have the head markings totally white. As occasional specimens from the Amazon basin, the Andean slopes in eastern Ecuador and in the valley of the Huallaga river also have all-white heads and palps, the identification of *erato* and *melpomene* by head color alone is not thoroughly reliable. The palps of *timaretus* are almost entirely black, there being only a very narrow outside line of white scales.

Table I. The Phenotypic Classification of the Color-pattern Components in *Heliconius erato* and *Heliconius melpomene*.

The code letters refer to the band shapes shown in Text-figures 2 and 3, with the exception of Y, which is an unfigured long band of medium width with a very variable appearance, and the Z-band, which is a broken yellow band with a narrow distal red transverse border. In the other columns, + means present, – means heterozygous, and v means variable. Brackets indicate an approximation only.

Geographical Locality	Named Form of *erato*	F/W Band				Dennis	Ray	Dorsal Yellow Bar	Ventral Yellow Bar	F/W Yellow Line	H/W White Margin	Iridescence 0-4	Named Form of *melpomene*
		Shape	Red	Yellow	White								
Western Ecuador	*cyrbius* *bellus*	A	+						+		+	4	
		A	+	v	+				+	v	+	3	*cytherus* *concinnus*
	diformatus	AB	+						+		+	4	
		AB	+	v	v				+	v	+	3	*modestus* *sticheli* *tenuistrigus*
Western Colombia	*venus*	B	+						+		–	4	
		B	+						+	v	–	2	*vulcanus*
Cauca valley	*molinus*	A	+						–			3	
	chestertoni							+	+			3	
	nocturnus							–?	–			3	
Magdalena valley, northern Colombia, Panama and Costa Rica	*colombinus* *antigonus*	C	+					+	+			1-2	
		C	+					+	+			1	*rosinus*
	guaricus	C	+						–			2	
		C	+						–			1	*euryas*
Panama and Costa Rica	*tristis*	CD	+						–			1	
	juno	BD	+						–			2	
Costa Rica and Mexico	*petiveranus* *demophoon*	D	+					+	+			0-1	
Eastern Colombia, Venezuela, Trinidad and the Guianas	*hydarus*	(E)	+					–?				1	
	vitellinus												
	adanus	E	+									1-2	
		E	+									1	*melpomene* *flagrans*

TABLE I. THE PHENOTYPIC CLASSIFICATION OF THE COLOR-PATTERN COMPONENTS IN *Heliconius erato* AND *Heliconius melpomene* (continued).

Geographical Locality	Named Form of *erato*	F/W Band				Dennis	Ray	Dorsal Yellow Bar	Ventral Yellow Bar	F/W Yellow Line	H/W White Margin	Iridescence 0-4	Named Form of *melpomene*
		Shape	Red	Yellow	White								
Central British Guiana	*magnificus*	(J)	+									3	
Northern Brazil	*rubrizonus*												
	viculatus	Y	+									0-1	
		Y	+	+								0	*luteipictus*
													eltringhami
													flavorubrus
	dryope	E	+			+						0-1	
		Y	+	v		+						0	*dianae*
													tyche
		Y	+	+		+						0	*maris*
	erythreus	EY	+			+	+					0-1	
	mixtus	E	+		+	+	+					0-1	
	tellus	E		+		+	+					0-1	
		Y	+	+		+	+					0	*punctarius*
	roseoflavus	EK	+	+		+	+					0-1	
	callistus												
	belticopis	K	+									0-1	
		ZK	+									0	*melpomenides*
													atrosectus
		ZK	+	+								0	*faivrei*
													lucius
													melpinus
													amor
													collis
	elimeus	K	+			+						0-1	
		ZK	+	+		+						0	*fascinatus*
													deinius
													faustinus
													negroidus
													dianides

TABLE I. THE PHENOTYPIC CLASSIFICATION OF THE COLOR-PATTERN COMPONENTS IN *Heliconius erato* AND *Heliconius melpomene* (continued).

Geographical Locality	Named Form of *erato*	F/W Band				Dennis	Ray	Dorsal Yellow Bar	Ventral Yellow Bar	F/W Yellow Line	H/W White Margin	Iridescence 0-4	Named Form of *melpomene*
		Shape	Red	Yellow	White								
Northern Brazil	*andremonus*	K	+			+	+					0-1	
	proteus	K	+	v	v	+	+					0-1	
	androdaixus	K	+	+		+	+					0-1	
		ZK	+	+		+	+					0	*hippolyte*
													athalius
													nigroidens
													augustus
													laurentinus
													agalopeius
													stygianus
													majesticus
													lucindellus
	amalfredus												
	helenae	K		+		+						0	
		K		+		+						0	*cybele*
													cybeleius
													eulalius
													funebris
													obscuratus
	vestus	K		+			+					0	
	erato												
	ledus												
	amazonus												
	hemicyclus												
	oberthuri												
	fuliginosus	K		+		+	+					0	
		K		+		+	+					0	*thelxiope*
													madeirus
													thelxiopeius
	callicopis	KN	+									0-1	
		ZN	+	+						+		0	*aphrodyte*
													karschi
													lucindus
	coralii	KN	+			+						0-1	
		ZN	+	+		+						0	*faustalius*
													elegantulus
	udalricus	KN	+			+	+					0-1	
		ZN	+	+		+	+					0	*rufolimbatus*

TABLE I. THE PHENOTYPIC CLASSIFICATION OF THE COLOR-PATTERN COMPONENTS IN *Heliconius erato* AND *Heliconius melpomene* (continued).

Geographical Locality	Named Form of *erato*	F/W Band											Named Form of *melpomene*
		Shape	Red	Yellow	White	Dennis	Ray	Dorsal Yellow Bar	Ventral Yellow Bar	F/W Yellow Line	H/W White Margin	Iridescence 0-4	
Northern Brazil	*constrictus*	KN		+		+	+					0	
		KN		+		+	+					0	*perchlorus*
													penelopides
	lativittus	LK		+		+	+					0	
		L		+								0	*mirabilis*
	cybelinus	L		+		+						0	
	estrellus												
	emmus												
	divus	L		+		+	+					0	
		L		+		+	+					0	*cognatus*
													rubrus
													agalope
													elevatus
	rothschildi	(L)	v	v	+	+	+					0	
	rosaceus	GL	+		+							0	
		GL	+		+							0	*coronus*
	ilius												
	ochraceus	GL		+		+	+					0	
Valleys of the eastern Ecuadorian Andes	*notabilis*	G	+		+							0	
		G	+	v	+							0	*plesseni*
													clytie
													rubricundus
													purus
													adonis
													fraternus
		G	+		+	+						0	*neipelti*
	radiatus												
	beatus	G	+	v	+	+	+					0	
		G	+	v	+	+	+					0	*isoldae*
													giselae
													iris
													adonides
													rubripictus

TABLE I. THE PHENOTYPIC CLASSIFICATION OF THE COLOR-PATTERN COMPONENTS IN *Heliconius erato* AND *Heliconius melpomene* (continued).

Geographical Locality	Named Form of *erato*	F/W Band				Dennis	Ray	Dorsal Yellow Bar	Ventral Yellow Bar	F/W Yellow Line	H/W White Margin	Iridescence 0-4	Named Form of *melpomene*
		Shape	Red	Yellow	White								
Valleys of the eastern Ecuadorian Andes	*feyeri*	LM	+		+	+	+					0	
		LM	+			+	+					0	*riffarthi*
	etylus	M		+		+	+					0	
		M		+		+	+					0	*ecuadorensis*
	agnatus	M	+			+	+					0	
		ELM	+			+	+					0	*rubrescens*
		M	+	+	+							0	*unimaculatus*
Upper River Huallaga	*favorinus*	H	+					+	+			0	
		H	+					+	+			0	*amaryllis*
	eratophilus	H	+			+	+	+	+			0	
		H	+			+	+	+	+			0	*melpophilus*
Upper River Ucayali-Perené	*microcleus*	I	+									0	
		I	+									0	*xenocleus*
		IJ	+									0	*confluens*
River Inambari	*amphitrite* *unipunctatus*	J	+									0	
		J	+									0	*euryades*
Southwestern Brazil	*cybelellus*	N		+		+						0	
	venustus *donatius* *telloides*	N		+		+	+					0	
		N		+		+	+					0	*vicinus* *penelope* *noctis*
	sanguineus *confluens* *henrici*	N	+			+	+					0	
		N	+	+		+	+	−	−			0	
		N		+		+	+	−	−	+		0	*margaritae*
		N	+			+	+	+	+			0	*penelemandae*
		N	+	+		+	+	+	+			0	*penelopeiae*
	leonorae	KL	+			+	+	−	−	+		0	
		ZK	+	+		+	+	+	+	+		0	*excellens*
		ZN	+	+		+	+	+	+	+		0	*amneris* *carneus*

TABLE I. THE PHENOTYPIC CLASSIFICATION OF THE COLOR-PATTERN COMPONENTS IN *Heliconius erato* AND *Heliconius melpomene* (continued).

Geographical Locality	Named Form of *erato*	F/W Band				Dennis	Ray	Dorsal Yellow Bar	Ventral Yellow Bar	F/W Yellow Line	H/W White Margin	Iridescence 0-4	Named Form of *melpomene*
		Shape	Red	Yellow	White								
Southwestern Brazil		ZN	+	+				+	+	+		0	*biedermanni*
		ZN	+					+	+	+		0	*amandae*
	speratus	N	+	+				–				0	
		N	+	+				–	–			0	*flammeus*
	diffluens	NF	+					+	+	+		0	
		NF	+	+				–	–	+		0	*amandoides*
	flavomixtus *anactorie*	NF	+	+		+	+			+		0	
	simplex	LF	+									0	
	palmatus	LF	+			+	+					0	
	anacreon	LF	+			+	+	+	+	+		0	
		LF	+			+	+	+	+	+		0	*curvifascius* *aidae*
	anacreonides *krugeri*	LF	+			+	+	–?	–?	+		0	
	amatus *athene*	LF	+					–	–	+		0	
		LF	+	+				–	–			0	*obscurifascius*
	ottonis	LF		+		+	+	+	+	+		0	
	differens	LF		+		+	+					0	
	celiae	F	+		+	+	+	–	–	+		0	
	anaitis	F	+				+	+	+	+		0	
	phyllidis	F	+					–	–	+		0	
Southern and eastern Brazil	*phyllis* *artifex*	F	+					+	+	+		0	
		F	+					+	+	+		0	*nannus* *besckei*
Hybridization zone in southern Colombia	*glaucinus*	CL	+						–			0	
		ZK	+		+							0	*annae*
		ZK	+	+								0	*parvus*
	problematus	CL	+			+	+	–?	–?			0	
		ZK	+		+	+	+					0	*gratiosus*
		ZK	+	+		+	+					0	*dione* *aurofasciatus*
		ZK	+	+		+	+	–				0	*paulae*
	meliorinus	CL		+		+	+	+	+			0	
	perplexus	NL	+			+	+	+	+			0	
	dignus	E	+					+	+			0	

TABLE I. THE PHENOTYPIC CLASSIFICATION OF THE COLOR-PATTERN COMPONENTS IN *Heliconius erato* AND *Heliconius melpomene* (continued).

Geographical Locality	Named Form of *erato*	F/W Band				Dennis	Ray	Dorsal Yellow Bar	Ventral Yellow Bar	F/W Yellow Line	H/W White Margin	Iridescence 0-4	Named Form of *melpomene*
		Shape	Red	Yellow	White								
Above 1,250 meters in the eastern Ecuadorian Andes		ZN		+								0	*timoretus*
		ZN	+	+			+					0	*virgatus*
													insoletus
													contiguus
													peregrinus
		ZN	+	+		+	+					0	*richardi*
		ZN	+	+						+		0	*erebius*
		ZN	+	+				+				0	*pluto*

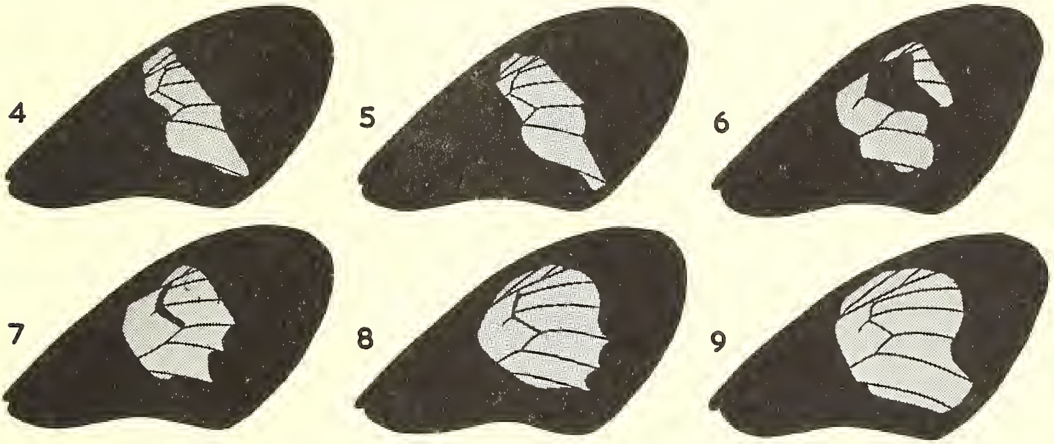
IV. GENETICAL EXPERIMENTS

In the context of these investigations the location of the New York Zoological Society's tropical research station in Trinidad is most fortunate, for while on the neighboring continent there is polymorphism and a high degree of variability, in Trinidad both *Heliconius erato* and *melpomene* are monomorphic and relatively constant. For this reason, and that of convenience, the Trinidad stocks have been used as a datum for comparison with material from western and eastern Ecuador. The experimental work, though performed as material was available, is here presented in two parts, the first involving *erato* and the second *melpomene.*

Heliconius erato

The named geographical subspecies used were *Heliconius erato cyrbius* Godart 1819 from Ecuador west of the Andes, *H. erato adanus* Seitz 1913 from Trinidad and *H. erato notabilis* Salvin & Godman 1868 from Ecuador east of the Andes. *Cyrbius* is figured in color in Seitz (1913) Plate 78 a; *notabilis* is figured in color in Eltringham (1916) Plate xii, figure 7; and though not known to be figured in color, *adanus* is similar to *dryope,* which is reproduced in Seitz (1913) on Plate 78 b, but without dennis.

As wild fertilized females of *cyrbius* and *notabilis* were collected on the first and subsequent trips respectively, it was possible to maintain



TEXT-FIGS. 4-9. Dorsal views of right forewings of laboratory-bred hybrids to show shape of bands. Text-fig. 4, F_1 of *cyrbius* × *adanus* (*erato*). Text-fig. 5, F_1 of *cytherus* × *flagrans* (*melpomene*). Text-fig. 6, F_1 of *notabilis* × *adanus* (*erato*), showing divided truncate band. Text-fig. 7, F_2 of *notabilis* × *adanus,* showing short semidivided truncate band. Text-fig. 8, similar F_2 showing short semidivided round tip band. Text-fig. 9, similar F_2 showing long undivided round tip band. Approximately 1½ times natural size.

pure stocks of both these Ecuadorian forms as well as the endemic *adanus*.

Field observations in the locality from which *cyrbius* was obtained showed that it is essentially monomorphic, though it is subject to minor variation in the width of the red forewing band, the width and density of the hindwing ventral yellow bar and the presence and development of the forewing costal spot. Variation in the width and density of the hindwing white margin is common only at the northern limits of its range.

In the locality from which *notabilis* was obtained it is also monomorphic, and though relatively constant in the size of the forewing double bands, it is variable in the relative proportions of red to white within them and in the distance by which they are separated. One example was taken in a total of about fifty in which the outer band is represented only by the inner half of the normally oval band, a condition referred to later as "truncate." Two such specimens are in the Joicey collection from the same locality.

SECTION A: WESTERN ECUADORIAN *cyrbius* AND TRINIDADIAN *adanus*

First Experiment: *cyrbius* × *adanus*

Reciprocal crosses were performed between the progeny of two wild fertilized *cyrbius* females brought back from western Ecuador and laboratory-reared Trinidadian stock *adanus*. Both sexes of all the progeny were reasonably uniform. The phenotypic characters of the parental and F_1 generations are summarized in Table II, though only only those characters in which the parents differed are included.

Expansion—The intermediate red band of the F_1 generation may be compared with those of the parents in Text-figures 2A, 2E & 4. It is interesting to notice that the F_1 band is similar to the wild form from northern Colombia (Text-fig. 2C), which is an intermediate geographical position.

The intermediate blue iridescence is also comparable with that occurring wild in northern Colombia, though the local wild variation never approaches the extremes shown by these experimental parents.

The hindwing ventral yellow bar is represented in the F_1 by an area which is similar in location to yellow bar, but which is differentiated from the normal brown ventral ground-color by appearing unusually shiny when examined by light reflected at low angles. It can be seen most easily by holding the insect upside down beneath a light source and altering the angle at which the wing is viewed. Museum collections contain many specimens which show this condition from Panama and from between the western and eastern cordilleras of northern Colombia, but beyond the extremities of the cordilleras it is a rarity. It is known also from Bolivia.

The hindwing white margin is represented in the F_1 by a peripheral border of intervenal terminal white scales which is variable in its completeness. In addition, there is a barely perceptible differentiation which is similar in texture to the F_1 hindwing bar over the border of the hindwing that bears the white margin in the Ecuadorian parent.

The light parts of the head pattern are principally yellow, but the palps grade from distally

TABLE II.

	PHENOTYPIC CHARACTERS		
	Parents		
	cyrbius	*adanus*	F_1 Generation
Ground Color	Bright blue iridescence	Dull blue iridescence	Intermediate blue iridescence
Hindwing White Margin	Well developed	Absent	Intermittent peripheral white scales and a differentiated border
Hindwing Ventral Yellow Bar	Well developed	Absent	Slight differentiation of ground color
Forewing Red Band	Narrow and short	Broad and long	Intermediate in both dimensions
Light Head Color	White	90% yellow 10% white	70% yellow 30% white
Forewing Costal Spot	Absent (in parents)	Present but variable	Variably present or absent

yellow to proximally 50% yellow and the latero-posterior aspects of the occiput are white. There is also a little more white on the vertex than is normal for the Trinidadian parent.

The forewing costal spot may be expressed in the F_1 generation. From the first experiment it may be concluded that:

1. There is no dominance between the broadness and narrowness of the *adanus* and *cyrbius* forewing bands.
2. There is no dominance between the longness and shortness of the *adanus* and *cyrbius* forewing bands.
3. There is no dominance between the blue iridescence of *cyrbius* and the dull iridescence of *adanus.*
4. The absence of hindwing white margin is semidominant to its presence.
5. The absence of hindwing ventral yellow bar is semidominant to its presence as expressed by *cyrbius.*
6. There is no dominance between the whiteness and yellowness of the light head markings of *cyrbius* and *adanus.*

Second Experiment: Backcrosses to *cyrbius* and *adanus*

In the backcrosses to the *cyrbius* and *adanus* stocks it was not possible to use the original male parents because they had died, but as both parental populations are reasonably constant the results yield good data. A summary of the phenotypic characters of the progeny is shown in Tables III and IV.

Expansion—Of interest is the fact that all characters may be expressed by both sexes, indicating that the sex-chromosomes are not involved and there is no evidence for sex-limitation.

It was not possible to score iridescence, for though there was considerable variation it seemed continuous within limits which were far less extreme than those of the parents.

The scoring of forewing band was similarly unsatisfactory, but this difficulty was overcome by measuring the length and breadth of the band in relation to wing width, though the results will be considered later together with those of the F_2 generation produced by the third experiment.

From the second experiment it may be concluded that the characters being studied are carried on the autosomes, so the sex of the progeny will not be recorded in succeeding tables.

Third Experiment: (*cyrbius* × *adanus*) × (*cyrbius* × *adanus*)

The phenotypic classification of the progeny of two brother × sister F_1 crosses is shown in Table V.

Expansion—The data from the backcrosses and from the F_2 generation are consistent with head color, hindwing ventral yellow bar and hindwing white margin each being controlled by a single pair of allelomorphs. The evidence for this is the near equality of the proportions of each class in the backcrosses and the reasonable approximation to 1:2:1 in the F_2. The chief discrepancy in this hypothesis is the apparent heavy weighting of the *cyrbius* class of head color in the backcross to *cyrbius* (Table III), but as the original male was not available and as only two backcrosses were made to *cyrbius* it is quite possible that the genetic background has shifted this difficult character towards the scoring of an excess of *cyrbius*-type individuals. Even so the data are not significantly inconsistent with the theoretical 1:1 proportions. Though unmeasured, the higher degree of variability of both hindwing ventral yellow bar and white margin in the F_2 suggests that modifiers are present in the *cyrbius* stock.

The data suggest that costal spot is inherited by a single pair of allelomorphs with presence of spot semi-dominant to its absence. Owing to the difficulty of identifying the heterozygotes with certainty, the spot class has been bulked for comparison with non-spot. The approximation to 3:1 ratios in the backcross to *adanus* and in the F_2 are consistent with this hypothesis if the *adanus* parents and the F_1 used were heterozygous for this character. This is plausible as non-spot occurred in the *adanus* stock at an unmeasured but low frequency. This potential heterozygosity in *adanus* accounts for interbrood variation in performance in the F_1 when crossed with the homozygous phenotypically non-spot *cyrbius.*

For the estimation of linkage, the number of recombinations of all the characters under consideration have been presented in Table VI.

The data give highly significant evidence for linkage between hindwing ventral yellow bar and hindwing white margin ($P = 0.001$). The maximum likelihood estimate for the crossover value is 14.4% ± 1.22% assuming no misclassification of the phenotypes. If there is a small degree of misclassification, then this estimate would be too high. This event is not unlikely, for the distinction between heterozygous bar and homozygous non-bar, and between heterozygous margin and homozygous non-margin, are in some instances extremely difficult to perceive.

There is no evidence for linkage between the genes controlling head color and either stripe or

TABLE III.

Brood	Parentage		Phenotype of Progeny								Total
			Hindwing Yellow Bar		Hindwing White Margin		Head Color		Forewing Costal Spot		
			cyr.	F_1	*cyr.*	F_1	*cyr.*	F_1	*cyr.*	F_1	
C14A	F_1 (*cyrbius* × *adanus*) ♂ × *cyrbius* ♀	♂	0	1	0	1	1	0	0	1	1
		♀	3	1	3	1	3	1	4	0	4
	Total		3	2	3	2	4	1	4	1	5
C13	F_1 (*cyrbius* × *adanus*) ♀ × *cyrbius* ♂	♂	4	5	4	5	6	3	(5)	(2)	9
		♀	3	5	3	5	6	2	(3)	(3)	8
	Total		7	10	7	10	12	5	(8)	(5)	17
Addition of C14A and C13			10	12	10	12	16	6	(12)	(6)	22

TABLE IV.

Brood	Parentage		Phenotype of Progeny								
C5A	F_1 (*cyrbius* × *adanus*) ♂ × *adanus* ♀		Hindwing Yellow Bar		Hindwing White Margin		Head Color		Forewing Costal Spot		
			adanus	F_1	*adanus*	F_1	*adanus*	F_1	Pres.	Absent	Total
		♂	4	8	4	8	7	5	9	3	12
		♀	4	4	2	6	4	4	7	1	8
		Total	8	12	6	14	11	9	16	4	20
C2	F_1 (*cyrbius* × *adanus*) ♀ × *adanus* ♂										
		♂	1	0	1	0	1	0	1	0	1

margin, nor is there conclusive evidence of linkage between costal spot and any other character studied, but the data are insufficient to deny that there is any linkage.

The forewing bands of the laboratory populations of the parental *cyrbius* and *adanus* stocks and those of their F_1 and F_2 progeny were measured for variation in width and length. The data, corrected for variation in wing size, are presented in Text-figs. 10 – 12 and 13 – 15, with similar data for *melpomene*. The most outstanding features of the F_2 data to be noticed at this stage are the unimodal distribution, the near complete absence of parental classes for both measurements and the location of the mode midway between those of the parental populations. The data from the back crosses (not figured) also produce a distribution with modes midway between those of the F_1 and appropriate parental population. Of interest too is the in-

TABLE V.

Brood	Parentage	Phenotype of Progeny											Total
		Hindwing Yellow Bar			Hindwing White Margin			Head Color			Forewing Costal Spot		
		cy.	F_1	*ad.*	*cy.*	F_1	*ad.*	*cy.*	F_1	*ad.*	Present (*ad.*+F_1)	Absent *cy.*	
C4	F_1 (*cyrbius* × *adanus*) ♂ × F_1 (*cyrbius* × *adanus*) ♀	3	8	7	3	9	6	4	10	4	15	3	18
C12	Replicate of C4	2	4	2	2	5	1	2	2	4	6	2	8
	Total	5	12	9	5	14	7	6	12	8	21	5	26

TABLE VI.

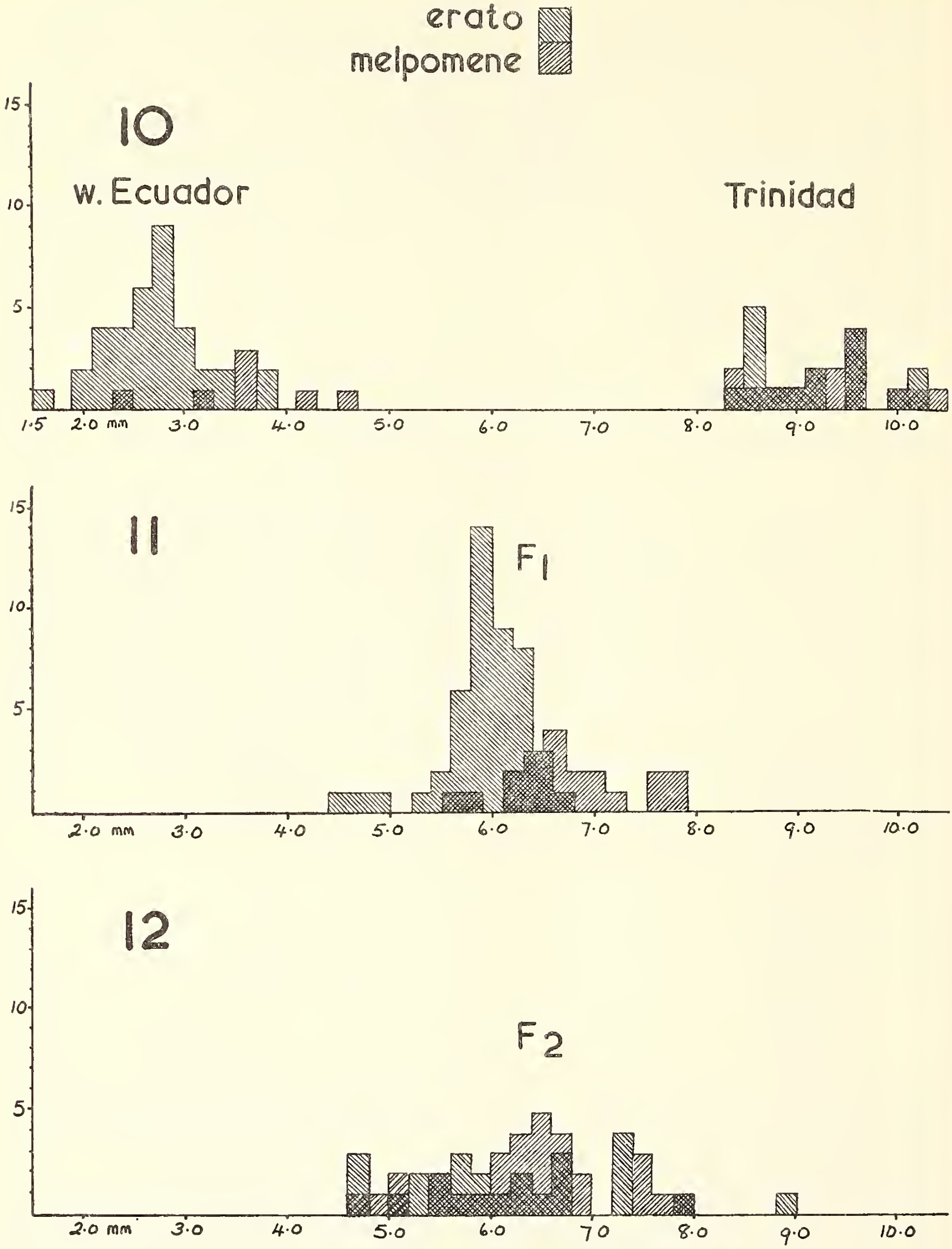
			Number of Recombinations of Characters in Progeny of *cyrbius* and *adanus*					
Brood	Parentage	Nos. of Specimens	Head Color & H/W Yellow Bar	Head Color & H/W White Margin	H/W Yellow Bar & H/W White Margin	Head Color & F/W Costal Spot	H/W Yellow Bar & F/W Costal Spot	H/W White Margin & F/W Costal Spot
C13	F_1 Backcross to *cyrbius*	17	9	9	0	2 (out of 13 specimens scored)	8(13)	8(13)
C14A	F_1 Backcross to *cyrbius*	5	3	3	0	2	3	3
C5A	F_1 Backcross to *adanus*	20	11	10	5	3	3	3
C4	(*adanus* × *cyrbius*)F_2	18	10	11	5	3	6	6
C12	(*adanus* × *cyrbius*)F_2	8	6	6	1	2	2	2

creased degree of variation in the F_2 measurements when compared with the F_1 or either parental stock. One may conclude that both the width and the length of the band in western Ecuador and Trinidad are either under polygenic control with little or no dominance or there are an approximately equally large number of dominant genes in each race. The gradual change in the dimension of the forewing band between western Ecuador and Central America and Trinidad is consistent with these data, though most sudden change takes place between western Ecuador and western Colombia.

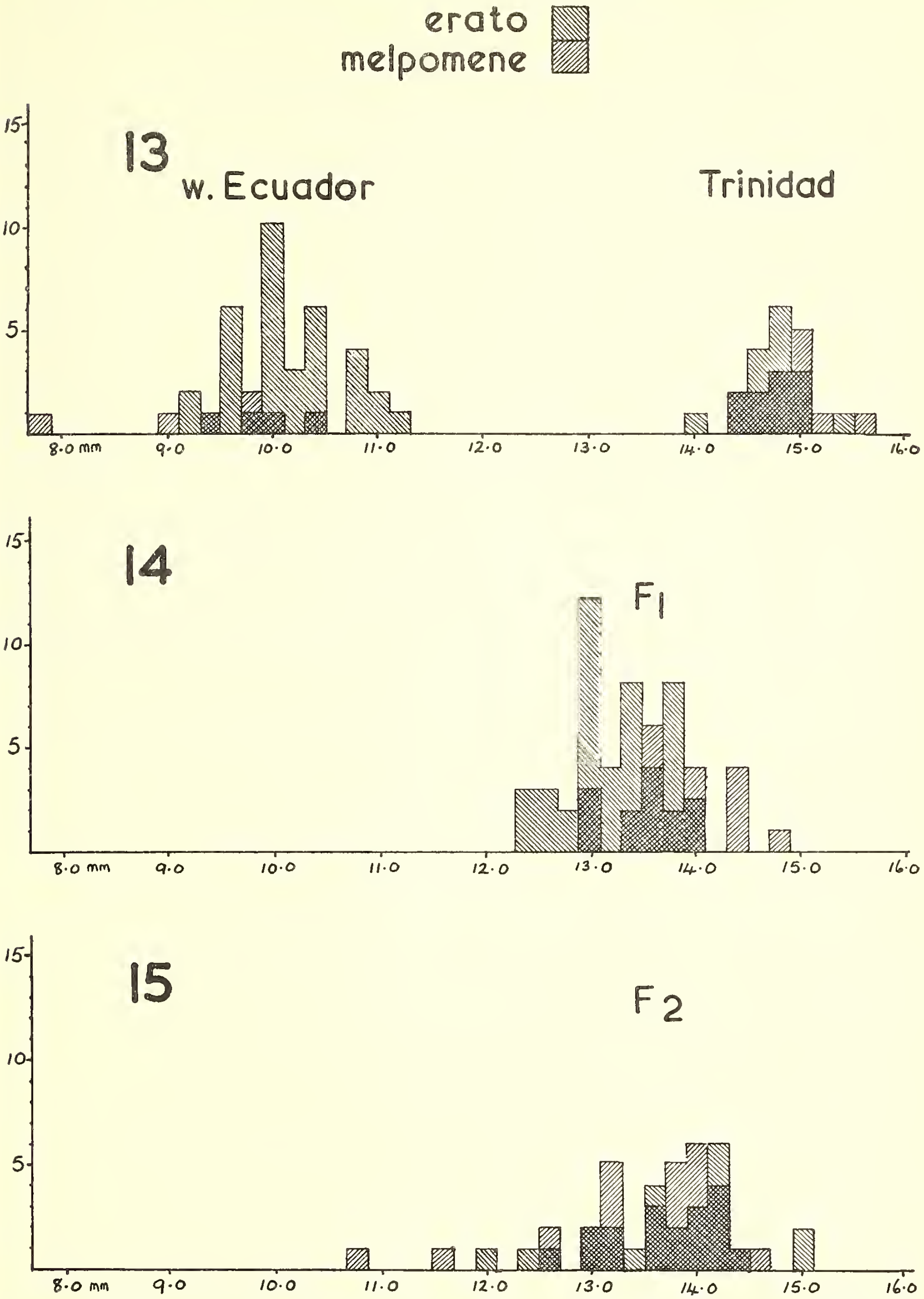
SECTION B: EASTERN ECUADORIAN *notabilis* AND TRINIDADIAN *adanus*

Fourth Experiment: *notabilis* × *adanus*

Reciprocal crosses were performed between pure stock males and females of *notabilis* from eastern Ecuador and *adanus* from Trinidad, but only fourteen F_1 individuals were produced because they were reared at a time of peak disease incidence. As the parental types differed only in respect of iridescence, forewing band and costal spot, these were the only characters studied and are recorded in Table VII.



TEXT-FIGS. 10-12. Frequency histograms demonstrating variation in band shape in *H. erato* and *H. melpomene*. Text-fig. 10—variation in band-width in the western Ecuadorian and Trinidadian parents; Text-fig. 11—variation in their F_1 progeny; Text-fig. 12—variation in the F_2 progeny. The vertical axes show numbers of individuals and the horizontal axes give measurements in mm. corrected to a standard wing-length of 16 mm.



TEXT-FIGS. 13-15. Frequency histograms demonstrating variation in band shape in *H. erato* and *H. melpomene*. Text-fig. 13—variation in band-length in the western Ecuadorian and Trinidadian parents; Text-fig. 14—variation in their F_1 progeny; Text-fig. 15—variation in the F_2 progeny. The vertical axes show numbers of individuals and the horizontal axes give measurements in mm. corrected to a standard wing-length of 16 mm.

TABLE VII.

	Phenotypic Characters		
	Parents		F_1 Generation
	notabilis	*adanus*	
Ground Color	Matt black	Dull blue	Just perceptible blue
Forewing Band	Red, white and yellow	Red	Red
	Double	Single	Variably intermediate
	Short	Long	Short
Forewing Costal Spot	Absent	Variably present or absent	Variably present or absent

Expansion—The iridescence of the F_1 generation was barely perceptible and in subsequent experiments was impossible to score, for the iridescence of *adanus* is only slight and the difference between the F_1 and either parent could not be detected with certainty.

The striking variability of the F_1 forewing band was due to variation in the shape of the tip and the degree of division of the band over the apex of the discal cell. The classification of the F_1 generation with respect to the tip shape and division of band is presented in Table VIII.

Though the F_1 forewing bands have been classified into "divided" and "semi-divided," it seems likely that with a larger sample the discretion of the classes would break down into more continuous variation. The semi-divided band has only a black line along the transverse vein at the apex of the discal cell (Text-fig. 8) but the divided band (Text-fig. 6) has a variable but appreciable quantity of black scales on the membrane immediately distal to the apex of the discal cell, which separates the band into two to a variable degree.

In all the 14 F_1 specimens the band length was only a little greater than that of the *notabilis* parent, and appreciably shorter than that of *adanus*.

The forewing bands of the F_1 were also divisible into a class in which the apex of the band

TABLE VIII.

Brood	Parentage	Phenotypic characters of F_1 progeny				
		Semidivided		Divided		Total
		Round	Truncate	Round	Truncate	
C18	Round tip *notabilis* ♀ × *adanus* ♂	0	2	4	3	9
C14B	Replicate of C18	0	0	0	3	3
C11	Round tip *notabilis* ♂ × *adanus* ♀	0	1	0	0	1
C5B	Replicate of C11	0	0	0	1	1
	Total	0	3	4	7	14

was rounded, as in Text-fig. 8, or one in which the tip was truncate, as in Text-fig. 7. The female parent of brood C 18 was phenotypically round though the progeny contained five truncate and four round tipped individuals. Bearing in mind that the female is the heterogametic sex in Lepidoptera, the presence of round and truncate bands in both sexes of the progeny of reciprocal crosses enables sex-linkage and sex-limitation to be disregarded. For this reason the sexes are not included in the tables.

The F_1 forewing bands were all red even though the band of *notabilis* has red, white and yellow scales.

Forewing costal spot is of variable occurrence in wild *notabilis* but was absent in the experimental parents. It was variable in both *adanus* and the F_1.

From the fourth experiment it may be concluded that:

1. Shortness in *notabilis* band is semidominant or dominant to longness in *adanus*.
2. The inheritance of division of band is complex.
3. Red is dominant to yellow and white.
4. There is no dominance between the dull iridescence of *adanus* and the matt black of *notabilis*.

Fifth Experiment: Backcrosses to *notabilis* and *adanus*

There were three successful backcross matings to *adanus*, the progeny of which are classified in Table IX, but only one adult emerged from the several attempted backcrosses to *notabilis*. In the backcrosses to *notabilis* and *adanus* stocks it was not possible to use the original male parents because they had died.

Sixth Experiment (*notabilis* × *adanus*) × (*notabilis* × *adanus*)

Again owing to disease only one offspring was produced; its characters are described in Table X.

Expansion—The near equality of the long and short-banded classes in the backcross progeny of broods C16, C6 and C7 suggests that only a single pair of allelomorphs is involved. The near-short length of the F_1 band and the recovery of the short band in the solitary backcross to *notabilis* (C19) and F_2 (C15) confirms that shortness is semidominant to longness.

The near equality of the truncate tip and round tip classes in the same backcross progeny are also consistent with control by a single pair of allelomorphs. The absence of round tip in any known *adanus* crosses not involving *notabilis*,

Table IX.

Brood	Parents	Progeny: Long Band, Round Tip	Progeny: Long Band, Truncate Tip	Progeny: Short Band, Round Tip	Progeny: Short Band, Truncate Tip	Total
C16	Round tip, semidivided F_1 (*notabilis* × *adanus*) ♂ × *adanus* ♀	7	8	8	4	27
C7	Same male as in C16 × *adanus* ♀	14	16	10	7	47
C6	Round tip, divided F_1 (*notabilis* × *adanus*) ♀ × *adanus* ♂	2	2	2	4	8
Total		23	26	20	15	84
C19	Truncate tip, undivided F_1 (*notabilis* × *adanus*) ♀ × Fully divided, round tip *notabilis* ♂	One ♀, double short band, truncate tip, red, white and yellow				

TABLE X.

Brood	Parentage	Phenotypic Characters of Progeny
C15	(*notabilis* × *adanus*) ♂ undivided round tip × (*notabilis* × *adanus*) ♀ undivided round tip	1 ♂, with double short band, round tip, red with a few yellow and white scales anteriorly

and the appearance of both round and truncate tip in the F_1 progeny, suggest the eastern Ecuadorian *notabilis* parents were heterozygous for this character. As they were phenotypically round-tipped the truncate-tipped condition must be recessive. Phenotypic truncate tip, which on present evidence is the homozygous recessive condition, is known from the parental *notabilis* locality but at very low frequency. In a sample of just over fifty wild specimens, only one was of this type. The frequency of the heterozygotes can be calculated as 27%, so the chance of heterozygotes being captured for establishing the breeding stocks was reasonable. There were very few emergences in the laboratory pure stock F_1 broods and no phenotypically truncate-tipped progeny were noticed.

In the backcross progeny C16, C7 and C6, all the long-banded forms, whether truncate or not, showed no division of the band at all (Text-fig. 9). All the short banded forms, both truncate and round, had the band divided by a variably broad transverse strip of ground color emanating distally from the apex of the discal cell (Text-figs. 7 & 8). This 100% association of long band and lack of division indicates either that both characters are under the control of one gene or that the two genes responsible are very closely linked. Judging by the variability in the performance of the factor for division in the F_1, it is semirecessive and probably multifactorially modified in the *notabilis* population.

An examination of the data from broods C16, C7 and C6 revealed no evidence of linkage between tip shape and band length.

The *adanus* and F_1 parents of Brood C7 each had a weak costal spot and the scored progeny segregated into 10 strong spot, 23 weak and 8 non-spot. This is consistent with a 1:2:1 ratio, suggesting that each parent was heterozygous for semi-dominant spot. Brood C16 was the progeny of a weak spot F_1 and a strong spot *adanus*, and all the 26 scored individuals had a variably strong costal spot. This would be the expected result of the homozygous dominant crossed with its heterozygote. These data support that derived from the *cyrbius* crosses, and suggest that costal spot in *erato* is controlled by a single pair of allelomorphs with spot semi-dominant to non-spot.

Heliconius melpomene

It was intended that exactly comparable crosses would be performed with *melpomene* as with *erato*, but due to deaths from virus disease these were not possible.

The subspecies used were strictly sympatric with the *erato* used and have been named *Heliconius melpomene cytherus* Hewitson 1869 from Ecuador west of the Andes, *H. melpomene plesseni* Riffarth 1907 from Ecuador east of the Andes and *H. melpomene flagrans* Stichel 1919 from Trinidad.

As only one wild male *cytherus* was brought back from the single trip to western Ecuador, it was not possible to maintain pure stocks of this form. In its locality it appears subject to variation only in the development of the white hindwing margin and the forewing white flecks.

SECTION A: WESTERN ECUADOR *cytherus* AND TRINIDADIAN *flagrans*

Seventh Experiment: *cytherus* × *flagrans*

Only one successful mating was achieved between the *cytherus* male and Trinidadian females. The phenotypic characters of the parental and F_1 generations are summarized in Table XI.

Expansion—The intermediate red band of the F_1 is subject to very little variation and may be compared with the parental bands in Text-figs. 3A, 3E & 5, and comparison with Text-fig. 3C shows that it is also similar to that occurring wild in northern Colombia, which is an intermediate geographical location.

The white flecks, which are characteristic of the *cytherus* forewing band, did not appear in any F_1 individuals.

The blue iridescence is also intermediate in intensity and again similar to that occurring in the valleys of northern Colombia, though, as in *erato*, the local variation there is far less than the difference between the experimental parental characters.

Table XI.

	Phenotypic Characters		
	Parents		F_1 Generation
	cytherus	*flagrans*	
Ground Color	Bright blue iridescence	Dull blue iridescence	Intermediate blue iridescence
Hindwing White Margin	Broad and complete	Intermittent peripheral scales only	Peripheral scales more complete and differentiated border
Hindwing Ventral Yellow Bar	Well developed	Absent	Slight differentiation of ground color
Forewing Red Band	Narrow and short	Broad and long	Intermediate in both dimensions
Light Head Color	5% yellow 95% white	95% yellow 5% white	90% yellow 10% white
Forewing Costal Spot	Absent (in parent)	Present	Present
Forewing White Fleck	Present	Absent	Absent
Forewing Yellow Line	Absent (in parent)	Absent	Absent

The hindwing ventral yellow bar is exactly comparable to that expressed in the *erato* F_1, in that it is an area which is similar in size and shape to bar, but differentiated from the ground color not by color but by reflectance.

The hindwing white margin is represented by an intervenal peripheral fringe of white scales which encroach onto the membrane to a variable but very limited extent, though the area occupied by the white margin in the Ecuadorian parent is differentiated by abnormal reflectance. As the parents from Trinidad have some peripheral scales of white, the F_1 with minimal white fringe is distinguishable from pure *flagrans* stock, only by the "shadow" of the broad margin.

The light markings of the head and thorax, when compared with *flagrans*, show only a slight reduction of yellow.

The forewing costal spot is fully expressed in the F_1 generation.

From the first experiment it may be concluded that:

1. There is no dominance between broadness and narrowness of the *flagrans* and *cytherus* forewing bands.

2. There is no dominance between the longness and shortness of the *flagrans* and *cytherus* forewing bands.

3. There is no dominance between the blue iridescence of *cytherus* and the dull iridescence of *flagrans*.

4. The absence of hindwing white margin is semidominant to its presence.

5. The absence of hindwing ventral yellow bar is semidominant to its presence as expressed by *cytherus*.

6. Preponderance of yellow head color is semidominant to preponderance of white head color.

7. The presence of forewing costal spot as expressed by *flagrans* is dominant to its absence.

8. The presence of forewing white fleck is recessive to its absence.

Eight Experiment: Backcrosses to *cytherus* and *flagrans*

Owing to disease the successful matings between the F_1 females and the original western Ecuadorian male parent produced only three progeny, the characters of which are recorded in Table XII.

TABLE XII.

Brood	Parentage		Phenotype of Progeny												
			F/W Yellow Line		H/W Yellow Bar		H/W White Margin		Fore-wing Fleck		Head Color		Costal Spot		
	F_1 (*cytherus* × *flagrans*) ♀		*cy.*	F_1	*cy.*	F_1	*cy.*	F_1	*cy.*	F_1	*cy.*	F_1	*cy.*	F_1	Total
F_1BX	× *cytherus* ♂	♂	1	0	1	0	1	0	1	0	1	0	0	1	1
		♀	0	1	0	1	0	1	0	1	0	1	0	1	1
M5	Replicate of F_1BX	♀	0	1	0	1	0	1	0	1	0	1	0	1	1

Ninth Experiment: (*cytherus* × *flagrans*) × (*cytherus* × *flagrans*)

The phenotypic classification of the progeny is presented in Table XIII. The small number in the backcross broods F_1BX and M5 are unfortunate but the F_2 data are sufficiently large, and bear a sufficiently close resemblance to a 1:2:1 ratio, for hindwing ventral yellow bar, hindwing white margin and proportions of white on the head each to be assumed to be controlled by a single pair of allelomorphs showing incomplete dominance. Costal spot, which is present in both *flagrans* and the F_1, bears a reasonable relationship to a 3:1 ratio in the F_2, which is consistent with the hypothesis that it is controlled by a pair of allelomorphs with presence of costal spot dominant. A similar situation pertains in forewing yellow line, except the absence of line is dominant and forewing fleck is probably also controlled by a pair of allelomorphs with its absence dominant. The male Ecuadorian parent must have been heterozygous for forewing yellow line, but being recessive it was not phenotypically expressed.

Measurements of the width and length of the forewing band in the parental, F_1 and F_2 generations were taken, corrected for a standard wing width, and plotted on the same histograms that portray the data in *erato,* (Text-fig. 10 — 15). Just as in *erato,* the unimodal curve of variation in the F_2, the greater variance in the F_2 when compared with the F_1 and the lack of parental classes of measurement in the F_2, all point to multifactorial control.

The probabilities for linkage between hindwing ventral yellow bar, hindwing white margin, white head color, costal spot, forewing yellow line and forewing white fleck have been calculated from the 2 × 2 tables constructed by considering the two phenotypes "west Ecuador" and

TABLE XIII.

Brood	Parentage	Phenotypic Characters of Progeny															
		F/W Yellow Line		H/W Yellow Bar			H/W White Margin			Costal Spot		Head Color			F/W Fleck		
		cy	F_1 & *fl*	*cy*	F_1	*fl*	*cy*	F_1	*fl*	*cy*	F_1 & *fl*	*cy*	F_1	*fl*	*cy*	F_1 & *fl*	Total
M4	(*cytherus* × *flagrans*) ♂ × (*cytherus* × *flagrans*) ♀	0	13	0	8	5	0	5	8	1	12	3	6	4	0	13	13
M8	Replicate of M4	3	13	4	9	3	4	9	3	3	13	6	5	5	3	13	16
F_1X	Replicate of M4	3	3	3	1	2	2	2	2	1	5	2	2	2	3	3	6
	Total	6	29	7	18	10	6	16	13	5	30	11	13	11	6	29	35

"not west Ecuador." This has been done to reduce errors caused by misclassification of phenotypes and the results are presented in Table XIV together with the maximum likelihood estimates for the crossover values. From the table it is clear that the genes controlling bar, margin, forewing line and fleck, lie between those controlling costal spot on the one hand and head color on the other. However, because of the large standard errors involved in the estimates and the disturbing effect of a small amount of misclassification of the phenotypes, the order of the genes, bar, margin, forewing stripe and fleck cannot be determined from the data with certainty. However, the most probable order on the chromosome is costal spot, white margin, yellow bar, fleck, forewing line, head color, with the genes controlling margin, bar, fleck and forewing line showing a very close linkage one with the other.

Tenth Experiment: (F_1 *cytherus* × *flagrans*) × *plesseni*

For reasons not understood the stocks of *plesseni* from eastern Ecuador never became established. Eggs were laid and larvae hatched but no adults were produced. However, though at the time it was considered a joke, a mating was effected between a *cytherus* × *flagrans* F_1 female and a wild double-banded *plesseni*. The five progeny are classified in Table XV.

Expansion—The forewing bands of all the progeny were short and more or less divided, as in the *erato* (*notabilis* × *adanus*) crosses. The progeny all had a costal spot, a preponderance of yellow on the head, and one specimen had both "shadow" ventral yellow bar and "shadow" hindwing white margin. The other specimens were as *flagrans* with neither. The data suggest that the

TABLE XIV.

Costal Spot	H/W White Margin	H/W Yellow Bar	F/W Fleck	F/W Yellow Line	Head Color
Costal Spot	19.1	21.5	18.8	28.6	38.0
p	.029	.044	.026	.195	.082
	H/W White Margin	6.3	7.4	7.4	38.9
	p	$10^{-6} \times 5.2$	$10^{-4} \times 1.26$	$10^{-4} \times 1.26$	.295
		H/W Yellow Bar	3.2	3.2	37.4
		p	$10^{-6} \times 4.31$	$10^{-6} \times 4.31$	.021
			F/W Fleck	6.7	22.7
			p	$10^{-4} \times 1.07$	.007
				F/W Yellow Line	33.0
				p	.063

TABLE XV.

Brood	Parentage	Phenotypic Characters of Forewing Band of Progeny				
		Semidivided		Divided		Total
		Round	Truncate	Round	Truncate	
SF_1	F_1 (*cytherus* × *flagrans*) ♀ × *plesseni* ♂	1	3	0	1	5

inheritance of red, division of band, and shape of tip, is in *plesseni* similar to that in the sympatric *erato notabilis*. The Z-band character which is peculiar to *melpomene*, appeared in one of the progeny and was probably carried in *plesseni* for it has not appeared in any *cytherus* × *flagrans* crosses.

V. DISCUSSION

In the preliminary morphological studies on *Heliconius*, which were reported in Emsley, 1963, it was deduced that *melpomene* is a more primitive species than *erato*, and further unpublished work on a revision of the genus supports this view. The description of the wild populations of *melpomene* shows that there is an incomplete circular cline commencing in western Ecuador, which passes around the spurs of northern Colombia and the highlands of British Guiana to the Amazon basin, and which terminates along the eastern slopes of the Andes and in southern and eastern Brazil. From any point in this area there are clines of minor variation which associate almost all the forms of *Heliconius* placed in the Melpomeneformes by both Stichel & Riffarth (1900) and Neustetter (1929). Direct confirmation of this widespread conspecificity has been obtained by the laboratory breeding of the geographically distant forms *cytherus*, *flagrans* and *plesseni* from western Ecuador, Trinidad and eastern Ecuador respectively. The only forms over which doubt exists are *heurippus*, *timaretus*, *besckei* and *tumatumari*.

Heliconius melpomene heurippus is a very constant form, confined to the valleys of the Guatiquía river above Villavicencio in eastern Colombia, and with which there are no known graded intermediates. There are, however, a few museum specimens from neighboring localities (*wernickei*, *emilius* and *rubellius*) which exhibit discrete intermediate characters between *heurippus* and the neighboring *melpomene rosinus*, and *Heliconius cydno*. These are considered to be wild hybrids, for the large red and yellow forewing band of *heurippus* could act as a courtship releaser for both the red-banded *rosinus* and the yellow-banded *cydno* (see Crane, 1955). The small differences that can be detected in the genitalia of *cydno*, *heurippus* and *rosinus* are not considered of importance for it has already been established (Emsley, *loc. cit.*) that there is no "lock and key" mechanism in *Heliconius* coition. Closely related species may or may not have identical genital valves, just as the genital valves may vary to a small degree over the range of a widely distributed continental species, or in isolated communities. The lack of graded intermediates between *heurippus* and the other morphs of *melpomene* can be explained by the altitudinal barriers presented by the head of the Guataquía valley and the uninhabited, inaccessible and uncollected nature of the territory to the southeast where intermediates could be expected to occur. Though the isolation of *heurippus* is comparable with that of the other forms of *melpomene* in the valleys of the eastern Andes, it is possible that it has become specifically distinct, but the structure and the coincident locality data of *emilius*, *rubellius* and *wernickei* leave no doubt that fertilization with *cydno* and *rosinus* has been effected. The detailed evidence for this will be presented in the revision of the genus.

It is now well established that the Amazon basin is occupied by yellow-banded *melpomene* which also exhibit dennis and ray. Along the slopes of the Andes, at about 850 meters at the equator, at 250 meters at 15° South and at pro rata altitudes in between, the yellow band gives way to one which is red or nearly all red and dennis-ray is lost. Only in the valleys of the Santiago and perhaps the Napo rivers is the yellow retained, then in a minimal condition. The occurrence of the yellow-banded dennis-rayed *timaretus* in the valley of the Pastaza and other rivers is inconsistent with this over-all generalization and is the only known exception. Specimens exhibiting variously graded intermediate characters are known from eastern Ecuador and Peru *(erebius, pluto)* which confirm the relatively wide distribution of the *timaretus* complex and associate it with undoubted members of *melpomene*. The scarcity of reliable and detailed locality data severely handicaps the interpretation of the situation, but as all the altitudes recorded are above those known for the non dennis-ray red-banded forms, it is most likely that *timaretus* is a highland form which maintains continuity around the spurs which sucessfully isolate the characteristic valley forms. There are no morphological grounds for regarding *timaretus* as a distinct species and there are no known sympatric species of *erato*.

In southern and southeastern Brazil the distribution of *besckei*, as judged by museum data, is discontinuous with that of *melpomene nannus*, so there is also a discontinuity in the distribution of the hindwing red arcs which are peculiar to *besckei*. The genital valves of *besckei* are a little different from those of *nannus* but the territory in the neighborhood of the Paraná river, which appears to separate them, is most inadequately collected. The two forms are so similar in morphological and wing-pattern detail that pending further data *besckei* will be considered conspecific with *melpomene*.

The status of *tumatumari* and *bari* are in doubt because they differ from other *melpomene* forms by the addition of a sub-apical forewing band and irregularities in the minor characters. They are known by very few specimens from the Guianas and are in other respects typically *melpomene*.

There can be no doubt now that all the forms of *Heliconius* listed by Neustetter under the Eratoformes are one species conspecific with *erato*. That *cyrbius, notabilis* and *adanus* from western, eastern Ecuador and Trinidad respectively, are conspecific has been demonstrated by breeding, and in the wild they are associated by being parts of an incomplete circular cline directly comparable with that pertaining in *melpomene*. All the known forms can be interconnected by graded series of intermediates though the rate of change is greater up the slopes of the eastern Andes than elsewhere.

The high degree of coincidence in the appearance and distribution of comparable wing characters in *erato* and *melpomene* is remarkable, for not only do the conspicuous components like wing band, yellow bar, iridescence and hindwing white margin match up in sympatric pairs, but the less conspicuous minor characters like head color, forewing yellow line, reduction of basal spots and loss of costal spot coincide too. With such a detailed and widespread similarity in color and pattern the possibility of this situation having arisen by chance alone can be disregarded, and the conception of mimicry must be accepted.

In the earlier paper on the Heliconiinae (Emsley, *loc. cit.*) it was shown that upon an examination of the species available in Trinidad it seemed likely that the genus *Heliconius* could be divided into three main stems. The most primitive group contained most of the members of the old genus *Eueides*, from which had arisen the *numatus, doris, wallacei, melpomene* association, and from which again had arisen the more advanced group containing *erato, ricini* and *sarae*. As *melpomene* and *erato* had so very many superficial characters in common it was considered that *erato* must have separated from *melpomene* and then given rise to the ancestor of the other species in its group. It is now possible to bring more detailed data to bear on the validity of the resemblances between *erato* and *melpomene* as a result of the recently completed breeding program.

Blue iridescence has been shown to be most probably under multifactorial control in both *melpomene* and *erato*. Considering the wide occurrence of iridescence in nearly related species of *Heliconius*, it may well be an ancestral character.

The shape of the forewing band of both species has been shown to be under very similar multifactorial control in western Ecuador and Trinidad. Turner & Crane (1962), in crosses with Guianese stock, identified a major locus for width of band in both *melpomene* and *erato*, and from the evidence in this paper it seems that there is a major factor or factors for shortness and division of band in both species in eastern Ecuador. The performance of tip control in eastern Ecuador also seems similar. The narrow-banded anomalies discovered by Sheppard (1963) are being re-examined by Turner, for it seems likely that there are multiple allelomorphs available at some loci. In both *melpomene* and *erato* red is dominant to both yellow and white. Though red and yellow occur in both species, yellow is the only forewing band color known to occur in the remainder of the genus, with the exception of *hermathenae*, and the monomorphic red-bands are all quite unlike any other band shapes in the genus. It seems more likely that yellow is the ancestral color, and red has been acquired independently in each species, than that red had been acquired by their mutual ancestor and independently they have reverted to yellow. If *tumatumari* is a good *melpomene* it supports this hypothesis, for the double nature of the yellow forewing band is very similar to that occurring in many of the other yellow-banded species of *Heliconius*.

Hindwing white margin is in both species controlled by a single pair of allelomorphs with its presence semirecessive to its absence. White margin is a feature of wide occurrence in near-relatives of both *melpomene* and *erato* which could have been present in the common ancestor.

Hindwing ventral yellow bar is again controlled by a single pair of allelomorphs with its presence semirecessive to its absence. There are no data on dorsal yellow bar, but from museum specimens there do appear minor differences in its genetic behavior, particularly between northern and southern *erato*. This character is of wide occurrence in the sylvaniforms, which are very close to *melpomene*, and in other closely related species. It does not occur in near-relatives of *erato*. There is evidence of close linkage between white margin and ventral yellow bar in both species ($p<0.001$). In *erato* the maximum likelihood estimate for the crossover value is 14.4% ± 1.22% whereas in *melpomene* it is 6.5% ± 2.8%. The figure for *erato* may well be lower in the absence of errors in scoring. This close agreement in linkage, taken together with the similarities in heterozygous expression and the presence of ventral yellow bar in all localities in both species where dorsal yellow bar occurs, suggests most strongly that it is an ancestral character.

Head color and costal spot in both *melpomene* and *erato* each appears to be under the control of a single pair of allelomorphs without dominance.

Forewing yellow line was not represented in the *erato* material studied and forewing white fleck does not occur in *erato,* but in *melpomene* both these characters were controlled by a single pair of allelomorphs with the presence of the character recessive to its absence.

In *erato* the data are imperfect and linkage can only be demonstrated between hindwing ventral yellow bar and white margin, though the data do not exclude the possibility of linkage between other studied characters. In *melpomene* there is evidence for linkage between all the wing pattern components with the exception of color, upon which there were no data. Though the data have not been presented, there is in *melpomene* a suggestion of linkage between some of the polygenes for band shape and yellow bar.

Accepting mimicry between these two species as a fact, the chance of two distantly related species having such a similar genetical constitution seems small, and the alternative is to concede that they are systematically closely related.

If one can accept that the similarities in *erato* and *melpomene* are the result of parallelism rather than convergence, those features which have a similar genetic performance in the two species were probably common to the mutual ancestor, whereas those that are different have had an independent origin or were represented in a polymorphic ancestral population. The data discussed above suggest that the mutual ancestor of *melpomene* and *erato* was a *Heliconius* with a blue iridescent ground color, yellow forewing band, a hindwing ventral yellow bar, a white margin to the hindwing and perhaps with a forewing yellow line and a dorsal yellow bar on the hindwing. The prevalence of basal red spots in the near relatives suggests that these were represented in a pattern not dissimilar to that of modern *melpomene.*

The substantial difference between the ray pattern in *melpomene* and *erato* could be explained if one postulates that the ancestral species was allopatrically polymorphic for this character, as *Heliconius doris* is at present. In *doris* there are two distinct ray patterns, a northern form which is almost exactly like the ray pattern of *melpomene timaretus* but with the bar of dennis incorporated proximally, and a southern pattern which is hardly distinguishable from that of *erato,* and six other species of *Heliconius.* In this theoretical discussion it is suggested that *melpomene* was derived from a population of the ancestral species which possessed the characters previously outlined and the *melpomene*-type ray; *erato* on the other hand evolved in isolation from another population of the ancestral species in which the *erato*-type ray was additional to the common characters. The occurrence of differences in the phenotypic expression of dennis presents a difficulty that can only be overcome by postulating a specific modification in the case of *melpomene,* for dennis as it occurs in *erato* is of wide occurrence in *Heliconius.* Some support is given to this hypothesis by the comparable linkage of dennis, ray and a band character in both species (Turner & Crane, 1962; Sheppard, 1963).

There are so many unknowns in the postulation of an evolutionary history for *melpomene* and *erato* that many biologists will feel it should not be attempted until more data are available, but the opportunities for the accumulation of more data are so few that it is likely that many years will elapse before really substantial evidence can be presented. The author feels, however, that if the hypothesis presented below provokes discussion among interested readers, new ideas may appear which would not otherwise have been considered. If this is so, then it is justified.

On the premise that the mutual ancestor was of the pattern described above, and the two characteristic ray morphs were allopatric and subsequently isolated from each other, then the two populations would have evolved along independent lines.

The author's knowledge of the geological and climatic history of the South American continent is insufficient to enter into discussion of where such geographical isolation took place or even how long ago. It is certain that the glacial periods have had a profound effect on the distribution of the Neotropical fauna, and one must not think of South America as having remained as is now during the evolution of even the geographical races of *melpomene* and *erato.*

It is suggested that the northern (?) population, which possessed the *melpomene*-type ray, evolved a red and yellow forewing band like that known in *heurippus* now. Such a red and yellow color combination would not have necessitated co-adaptation of the courtship releaser mechanism. The evolution of dominance of red together with the evolution of red as a courtship releaser would present a situation similar to that which we believe currently pertains in the peripheral region of South America. One must assume that the factors that have prevented *melpomene* becoming an all-red-banded species were the same then as they are today. The author's experience on the eastern slopes of the Ecuadorian Andes may be important here.

Puyo is located at an altitude of just under 1,000 meters on a tributary of the River Pastaza, and the only morphs of *melpomene* and *erato* that occur there are black with a double red and white forewing band. They confine themselves principally to streams, paths and glades in the interior of the forest and only occasionally do either of them fly beyond forest margins. Their light and dark pattern and erratic flight make their position very hard to estimate when trying to catch them, even though they do not fly very powerfully. One cannot help but notice the almost total absence of "tiger"-patterned butterflies. There are very few danaids, and those there are have a single broad orange forewing band, with no suggestion of yellow and black, and they confine themselves to the interior of the forest. There are few *Heliconius isabellus* or near relatives, and very few Sylvaniformes and those that are present are patterned like the danaids with which they fly.

No more than 40 km. east, and after a descent of only 100 meters, the scene changes abruptly and spectacularly. "Tiger"-patterned Sylvaniformes, *isabellus,* danaids and ithomiines abound on the vergeside flowers, and all the *melpomene* and *erato* seen are with a double-yellow or white forewing band and dennis-ray. They fly in exposed positions and with a much more purposeful manner than those from the higher altitudes. The visual effect of a "tiger"-patterned danaid and a yellow-banded dennis-rayed *Heliconius* is, to human eyes, very similar. From the body there are in successive order on each side, a broad orange-red band, a yellow band and a black tip. The author had considerable difficulty in distinguishing them in flight.

This experience was so impressive that it may well be an effect which has governed the distribution of yellow band in *erato* and *melpomene* for a considerable period of their history. One would infer that where danaids and the less diaphanous ithomiines are the ecologically dominant groups, selection for yellow-band and dennis-ray is stronger than any other force. Where the populations of these proved distasteful groups are low, as in Trinidad and above 900 meters in eastern Ecuador, selection for yellow band and dennis-ray is low and courtship selection for red, together with dominance of red over yellow, maintains an all-red population. In intermediate areas like the Guianas, southern Colombia and Bolivia where the two types of pattern meet, there is a region of polymorphic hybridization which is kept in a state of dynamic equilibrium by the opposing forces on either side of the zone.

If the area in the Amazon basin currently occupied by yellow-band and dennis-ray was in the past a little smaller than it is now, then the red-banded forms could have occupied the foothills of the Andes quite broadly, and with an increase in the range of the yellow band dennis-ray, presumably on account of the increase in the range of the danaids, the red-banded non-dennis-ray forms would have been cut off in the river valleys of the eastern Andes, which is the situation in which we find them today.

The most obvious deficiency in our knowledge in this hypothesis is the quantitative estimate of danaid and other model populations in the region inhabited by *erato* and *melpomene.* This could be obtained by statistically acceptable sampling in a small number of carefully chosen localities on the boundaries of the two zones.

Prior to the segregation of the yellow band dennis-ray and red band non-dennis-ray types, one must assume that the *erato*-rayed population, isolated in the south, rejoined its parent population after specific barriers had become erected.

During the evolution of red forewing band in *melpomene* one must postulate an analagous mutation in *erato,* and also assume the evolution of courtship selection for red. The behavior of red and yellow in combination in *melpomene* and *erato* supports this suggestion, for in the northern part of their range all the red and yellow combinations known in *melpomene* are of the *heurippus* type of interface, whereas the only known type of interface in *erato* is one in which the two colors intermingle over a large area. It is worth noting here that the *heurippus*-type interface also occurs in *timaretus,* which also has the most *doris*-like ray known in *melpomene.* It is the only *melpomene* known to occur in the absence of *erato,* and should perhaps be considered a relic.

Once *erato* had evolved a red forewing band it would have been under the same type of selection forces as *melpomene,* and having a similar basic appearance and genetic constitution, it has maintained a step by step mimetic relationship.

There is one further complexity. There are at present no records of *melpomene* from the east coast of Brazil. If this distribution is the real situation and is one which has persisted during a substantial part of the evolutionary period under discussion, then it is possible that red-banded non-dennis-ray *erato* extended its range around the coast and the Brazilian highlands into southwestern Brazil where it would have met yellow-banded Amazonian *melpomene* and *erato.* The *erato* would have interbred and the red band would have spread until the polymorphic equilibrium was reached, somewhat as it is now in eastern Bolivia. The *melpomene,* however, would have had only yellow band and been unable to enter a mimetic relationship with *erato* in the red zone without an independent mutation for red band color, the *heurippus* band being confined to the north. The successful mutation of a second form of red band in this southern locality could explain why the red yellow interface of southern *melpomene* are not of the *heurippus* type but with yellow and red scales broadly intermingled over a substantial area.

A minor supporting feature is the peripheral distribution of dorsal yellow bar, and the relic retention of white margin at the western extremity of the range of the species. The ventral red raylets on the ventral surface of the hindwing of southern *erato* may be a relic of a line of dots emanating from the basal spots as also occurs in *sarae.* This feature would not have been in southern *melpomene,* as the hypothesis supposes that *erato* arrived there second, so it is not unexpected to find a new feature matching it in the red arcs of *besckei.*

One of the embarrassing aspects of this mimetic situation is the degree of detail in the similarities between the two species. Not only are the general proportions, locations and color of the patterns similar, but the shape of the bands corresponds in detail and the variations of even small characters like costal spot are comparable. The evidence from avian predators of *Heliconius* (Brower, Brower & Collins, 1963) does not suggest that they can appreciate detail of this magnitude, at least not under experimental conditions where one would expect them to have more opportunity for careful scrutiny than in the wild. In view of the close likeness between the genetic constitution of the two species, it seems likely that many of the detailed resemblances are coincidental. The selection for a major pattern-component, whether for the simulation of each other or of a third party, would effect similar changes in the genotype of both species. The genetic units which control the composition of the color-pattern can only respond to a given situation in a basically similar way. It has also to be remembered that the eggs, larvae and pupae all have the same genes, so a character of selective advantage in one stage may produce a neutral but visible effect in another, etc.

The question of whether *erato* and *melpomene* are Batesian or Müllerian mimics has been substantially answered by the data obtained by Brower, Brower & Collins (*loc. cit.*) who found that though both can be classed as unpalatable, *melpomene* was more frequently rejected than *erato.* Whether the relationship has always been Müllerian is a question that cannot be answered, but it is easier to believe that it has than that it has not. If the ancestor was already distasteful before the speciation in which we are interested took place, then the relationship has undoubtedly always been Müllerian. If, however, the common ancestor was palatable, and say *melpomene* was the first to acquire distastefulness, it is difficult to understand how *erato* could have acquired it except by a similar one-step change. Rothschild's view (1963) on how Müllerian mimicry can evolve gradually from a Batesian relationship is interesting. Her view, in essence, is that while the imperfect mimic in a Batesian relationship is under strong selection for good resemblance, once an adequate degree of similarity has been obtained, though the likeness should improve, there will be additional selection for distastefulness. However, unless the insect survives the attack, and is reproductively viable, selection on palatability alone cannot operate. The predator must learn to associate its experience with a visual image which it can remember, so it is not the attacked insect that escapes but another similar one. Unless there is a visually perceptible character in association with unpalatability, though the population as a whole may benefit from the predator's experience, there is no selective advantage gained by the unpalatable members only.

Hitherto, birds have been the only class of predator considered, but the number of eyewitness accounts of birds attacking *Heliconius* is very small, though collectors' fastidiousness will have probably excluded such attacked insects from their collections. In Colombia several terrestrial burrows were found in the forest, at the entrance of which were many *Heliconius* remains. The occupants of the burrows are unknown. Lizards have good color vision and members of the genus *Ameiva* have been an occasional nuisance in the mating cages, for once they have gained entrance, they climb the wire-netting and lie in wait by the floral food supply, and snap at butterflies as they settle.

VI. Conclusions

1. The Eratoformes and the Melpomeneformes, as catalogued by Neustetter (1929), are each a single species, *Heliconius erato* and *melpomene* respectively, which exhibit pronounced geographical variation.

2. The two species are grossly sympatric and maintain a close mimetic relationship over their whole range.

3. The two species are held to be systematically closely related on account of the very similar genetic performance of the characters studied. Experiments between material from western Ecuador and Trinidad have shown that in both

species the shape of the forewing band and the intensity of the iridescence are under multifactorial control. The inheritance of hindwing ventral yellow bar, hindwing white margin, head color and the presence of costal spot is in both species controlled by a single pair of allelomorphs with similar dominance and similar heterozygotic expression. There is evidence of close linkage between ventral yellow bar and hindwing white margin in both species. In *melpomene,* all the pattern components were shown to be linked, but though no such linkage was demonstrated in *erato,* the possibility of such linkage cannot be denied. Though no comparison could be made, the forewing yellow line and forewing fleck of *H. melpomene* in western Ecuador also appear to be under the control of a single pair of allelomorphs.

Experiments between material from eastern Ecuador and Trinidad show that in *erato* the outer band tip-shape is controlled by a single pair of allelomorphs and there is a major factor, or pair of very closely linked factors, of considerable penetrance controlling shortness and division of the forewing band. Though the evidence is inconclusive, it is likely that the situation in *melpomene* is similar.

4. From the distributional data, it is shown that the yellow-banded dennis-rayed non-yellow-barred forms of both species in the Amazon basin are surrounded by red-banded yellow-barred non-dennis-rayed forms. The view is held that the stability of the Amazonian forms is maintained by strong selection for resemblance to danaids and other distasteful butterflies whose wing pattern from apex to base forms the series black-yellow-orange.

5. It is suggested that the stability of the peripheral red-banded non-dennis-rayed populations is maintained by the importance of red as a courtship releaser, the dominance of red over yellow and white and the weak selection for resemblance to the less abundant danaid-type models.

6. The opinion is held that the polymorphism in the Guianas, east-central Colombia, the eastern slopes of the Andes and in Bolivia, is a dynamic equilibrium brought about by the opposing influences mentioned in 4 and 5 above.

7. The exceptionally close similarity between sympatric forms of *melpomene* and *erato* is deemed to be due primarily to their close systematic relationship. Their genetic constitution is so similar that having been subjected to common selective forces since becoming specifically distinct, they have been able to respond to selection only in a very similar way. It is thought that in the Amazon basin there is a triangular mimetic relationship between the strongly distasteful models at one corner and *erato* and *melpomene* at the other two, so each has effected a compromise in attaining the pattern exhibited by the other two competitors. Beyond the dominating influence of the Amazonian danaids there is only a dual Müllerian relationship between *erato* and *melpomene.*

VII. SUMMARY

1. The color-pattern components of the wings of *Heliconius erato* and *H. melpomene* are described and their distribution recorded.

2. It is shown that both the major and minor color-pattern components are comparable and similar components occur only sympatrically in the two species.

3. By laboratory breeding it was established that *H. erato cyrbius, adanus* and *notabilis* are conspecific, and *H. melpomene cytherus, flagrans* and *plesseni* are also conspecific. From the data and a survey of museum material, it is inferred that all named forms catalogued by Neustetter (1929) in the Eratoformes and Melpomeneformes are members of the two species *erato* and *melpomene* respectively.

4. The genetical data presented indicates a close similarity in the performance of the genes controlling forewing band, ventral yellow bar, hindwing white margin, head color and forewing costal spot in populations of both species from western Ecuador and Trinidad. These similarities in genetical constitution suggest that the two species are systematically closely related.

5. The evolution of the color patterns in the two species is discussed with particular reference to mimicry.

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APPENDIX: Brood-rearing Data, Showing the High Incidence of Mortality Which Is Almost Entirely Attributed to Virus Disease

Brood	Parentage	Duration of Oviposition in Days	Numbers of Eggs Laid	Numbers of Larvae	Numbers of Pupae	Numbers of Adults
	Original *cyrbius* females	21	19	19	10	9
	Original *notabilis* females	14	20	20	9	5
	cyrbius × *cyrbius* (stock)		60	51	19	17
	notabilis × *notabilis* (stock)		201	176	31	16
	adanus × *adanus* (stock)		65	64	15	11
Pb1	*cyrbius* × *adanus*	108	103	94	28	24
Pb2	*cyrbius* × *adanus*	133	117	109	27	26
Pa1	*cyrbius* × *adanus*	138	235	211	52	40
C17	*cyrbius* × *adanus*	64	84	61	30	28
C12	(*cyrbius* × *adanus*) F_2	16	19	19	8	8
C4	(*cyrbius* × *adanus*) F_2	54	60	58	23	18
C3B	(*cyrbius* × *adanus*) F_2	53	22	6	0	0
C2A	(*cyrbius* × *adanus*) F_2	8	18	16	0	0
C19	(*cyrbius* × *adanus*) F_2	6	3	3	0	0
F_1X2	(*cyrbius* × *adanus*) F_2	33	37	30	0	0
C2	(*cyrbius* × *adanus*) × *adanus*	20	36	25	1	1
C5A	(*cyrbius* × *adanus*) × *adanus*	96	104	98	25	20
BXWE	(*cyrbius* × *adanus*) × *cyrbius*	52	86	63	0	0
C13	(*cyrbius* × *adanus*) × *cyrbius*	142	156	148	29	17
C14A	(*cyrbius* × *adanus*) × *cyrbius*	101	98	70	12	5
C18	*notabilis* × *adanus*	61	71	64	12	9
C5B	*notabilis* × *adanus*	31	44	33	2	1
C14B	*notabilis* × *adanus*	24	25	8	6	3
C11	*notabilis* × *adanus*	5	6	6	1	1
C15	(*notabilis* × *adanus*) F_2	21	23	10	9	1
C7	(*notabilis* × *adanus*) × *adanus*	96	84	72	54	47
C6	(*notabilis* × *adanus*) × *adanus*	30	50	48	10	8
C16	(*notabilis* × *adanus*) × *adanus*	53	65	60	38	27
C19	(*notabilis* × *adanus*) × *notabilis*	24	34	20	1	1
Ma	*cytherus* × *flagrans*	99	248	175	52	44
M4	(*cytherus* × *flagrans*) F_2	74	142	83	19	13
M6	(*cytherus* × *flagrans*) F_2	6	4	0	0	0
M8	(*cytherus* × *flagrans*) F_2	25	96	50	20	16
F_1X	(*cytherus* × *flagrans*) F_2	41	77	36	8	6
TX2	(*cytherus* × *flagrans*) × *flagrans*	38	40	36	0	0
F_1BX	(*cytherus* × *flagrans*) × *flagrans*	36	98	87	3	2
M5	(*cytherus* × *flagrans*) × *cytherus*	10	5	3	1	1
SF_1	(*cytherus* × *flagrans*) × *plesseni*	21	36	29	6	5

SUMMARY

Eggs Laid	Eggs Hatched	Larvae Pupated	Pupae Emerged	% Insects Successfully Reared of Eggs Laid
2691	2161	561	430	16.0%

EXPLANATION OF THE PLATE

PLATE I

The color components of the wing patterns of *Heliconius erato* and *melpomene*. Approximately twice natural size.

FIG. 1. Dorsal view of a right forewing illustrating black ground color; double yellow band; dennis; and fully developed forewing yellow line.

FIG. 2. Dorsal view of a right forewing illustrating blue iridescent ground color; short narrow red band as it occurs in *cyrbius;* and yellow basal spot.

FIG. 3. Dorsal view of a right hindwing of a male *melpomene* illustrating bar of dennis; white margin as it occurs in *cytherus;* and black ground color.

FIG. 4. Dorsal view of a right hindwing of a female *erato* illustrating blue iridescent color; dorsal yellow bar; and white margin as it occurs in *cyrbius.*

FIG. 5. Dorsal view of right hindwing of male *erato* illustrating ray on black ground color.

FIG. 6. Dorsal view of right hindwing of female *melpomene* illustrating non-dennis ray on black ground color.

FIG. 7. Ventral view of right hindwing of *erato* illustrating brown ground color; yellow bar; yellow costal streak; red basal spots; apical cream rectangles; and raylets.

FIG. 8. Ventral view of right hindwing of *melpomene* illustrating brown ground color; yellow bar; yellow costal streak; red basal spots; and submarginal red arcs with their apical cream rectangles.

FIG. 1

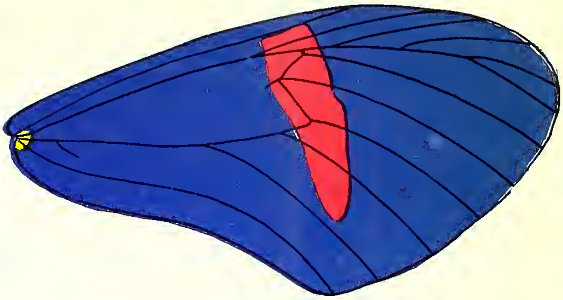

FIG. 2

FIG. 5

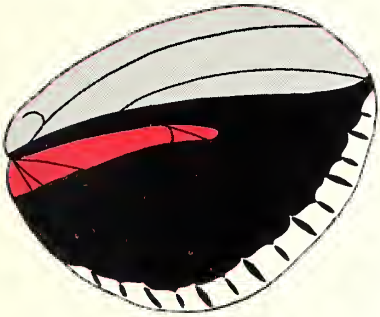

FIG. 3

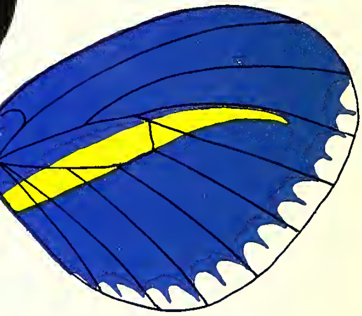

FIG. 4

FIG. 6

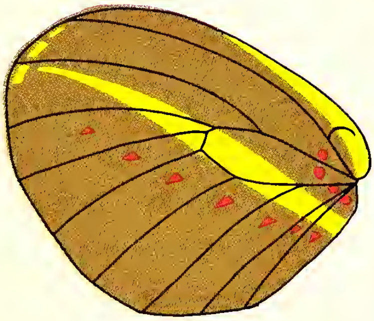

FIG. 7

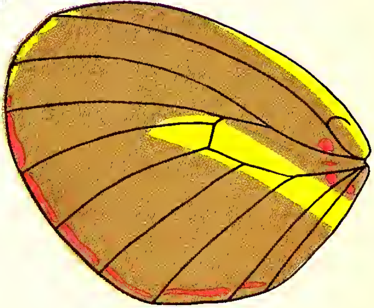

FIG. 8

THE GEOGRAPHICAL DISTRIBUTION OF THE COLOR-PATTERN COMPONENTS OF HELICONIUS ERATO AND HELICONIUS MELPOMENE WITH GENETICAL EVIDENCE FOR THE SYSTEMATIC RELATIONSHIP BETWEEN THE TWO SPECIES

EXPLANATION OF THE PLATE

PLATE II

The geographical distribution of the more conspicuous color components in Heliconius *melpomene* and *erato*. Regions not known to be inhabited by either species are shown in black.

FIG. 1. The distribution of red and yellow forewing bands. Areas inhabited by red banded forms are shown in red, those inhabited by yellow banded forms are shown in yellow.

FIG. 2. The distribution of dennis-ray without yellow bar (shown in red), non-dennis non-ray without yellow bar (shown in green), and non-dennis non-ray with ventral or dorsal and ventral yellow bar (shown in yellow).

FIG. 1

FIG. 2

THE GEOGRAPHICAL DISTRIBUTION OF THE COLOR-PATTERN COMPONENTS OF HELICONIUS ERATO AND HELICONIUS MELPOMENE WITH GENETICAL EVIDENCE FOR THE SYSTEMATIC RELATIONSHIP BETWEEN THE TWO SPECIES

INDEX

Names in **bold face** indicate new genera, species or subspecies; numbers in **bold face** indicate illustrations; numbers in parentheses are the series numbers of papers containing the plates listed immediately following.

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