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EXTERNAL MORPHOLOGY OF ADULT AND COPEPODID STAGES OF *DIAPTOMUS CLAVIPES* SCHACHT 1897

By

Ahmad Kamal and Kenneth B. Armitage

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External Morphology of Adult and Copepodid Stages of *Diaptomus clavipes* Schacht 1897

AHMAD KAMAL* AND KENNETH B. ARMITAGE
Department of Zoology, The University of Kansas, Lawrence

INTRODUCTION

In development, many copepods pass by molting through five or six naupliar and six copepodid stages, the last of which is the adult. Although the general structure of the larval stages is known (Grandori, 1912; Gurney, 1931; Ravera, 1953; Wilson, 1959; Wilson and Yeatman, 1959), few studies have examined the detailed morphology of fresh water Copepoda.

The most recent extensive description of all stages of development is that of *Diaptomus siciloides* Lilljeborg 1889 (Comita and Tommerdahl, 1960). Because *Diaptomus siciloides* frequently occurs in the same body of water with *Diaptomus clavipes* (Armitage, 1961), the latter was chosen for morphological study. Of special interest is the examination of structural diversity characteristic of speciation in *Diaptomus.* The study emphasizes the external anatomy of the adult and copepodid stages which differs from published descriptions of other diaptomids. Detailed comparisons are not presented because of the intensive tabulation required and because sufficient data are not available at this time to warrant such a comparison.

METHODS

The specimens used for this study were collected from ponds at the University of Kansas and preserved in 10% formalin. Each copepodid stage was dissected with microneedles under a binocular microscope and the appendages were mounted in glycerine or in C. M. C-S non-resinous stain mountant (Turtox). Often several specimens were dissected in order to study structural detail.

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* Present address: Department of Zoology, University of Cincinnati.

Drawings were made only for those structures which are important in the identification of the copepodid stages. All drawings were made by tracing the image projected on paper by a B & L VH microprojector. Each copepodid stage is represented by C followed by the roman numeral for that stage; e.g., CI=Copepodid stage one. All body measurements were made with an ocular micrometer and include the whole organism from the rostrum to the distal tips of the caudal rami; caudal setae are excluded.

ANATOMY OF COPEPODID STAGES

Stage I. CI larvae are about 0.46 mm long and are composed of 6 segments. Antennules extend nearly to the tip of the caudal rami. Legs 1 and 2 are functional but the rami are unsegmented. Leg 3 is present as a bilobed rudiment. The abdomen is composed of a single segment. There are no medial or lateral hairs on the caudal rami.

Stage II. The body is about 0.58 mm long and composed of 7 segments. Antennules extend to the tip of the caudal rami. Leg 4 is bilobed.

Stage III. The eight-segmented body is about 1.04-1.08 mm long. This stage has 4 pairs of swimming legs and a pair (the 5th) of bilobed rudiments. The abdomen is composed of 2 segments.

Stage IV. The body-length of the male is about 1.21 mm and the female about 1.40 mm. The body of each sex is composed of 9 segments, the abdomen of 3 segments. Sexes at this stage can be distinguished not only by size but also by the structure of the fifth swimming leg.

Stage V. The body-length of the male is 1.56-1.64 mm and the female about 1.76 mm. The body is composed of 10 segments. In the male the right fifth leg is distinct from the left. The total number of segments in the abdomen is 3 in the female, and 4 in the male. The medial surface of each caudal ramus has a comb-like arrangement of hairs, but the outer surface does not.

Stage VI. The body-length of the male is 1.28 to 2.2 mm, whereas the adult female is typically larger, 1.57 to 2.5 mm. The right antennule of the male is geniculate. No important sexual differences were noted in the structure of the left antennule, antennae, mandibles, maxillae, maxillipeds and swimming legs. The abdomen is composed of 5 segments in the male and 3 in the female.

ANATOMY OF THE APPENDAGES

ANTENNULE (TABLE I)

Stage I. The antennules extend nearly to the tips of the caudal rami. The antennule is composed of 10 apparent segments, but the sutures are not distinct (Fig. 1). The fused segments are indicated in Table I by brackets.

Table 1. Armature of Copepodid Antennules. Entries refer to the kind of armature as follows: a, aestheta; s, seta; sp, spines; rs, rudimentary seta; ns, no seta; ls, long seta; vls, very long seta; stsp, stout spine. Brackets indicate the fusion of segments. Length of seta is defined as follows: a seta (s) is one which does not extend beyond the distal margin of the next distal segment; a long seta (ls) is one which extends to any length beyond the proximal and distal margin of the second distal segment; a very long seta (vls) is one which extends beyond the second distal segment.

	I	II	III				V		VI	
No. of Segments	Right 11	Right 18	Right 23	Left 23	Right ♀ 25	Left ♂ 25	Right ♂ 25	Left ♂ 25	Right ♂ 25	Left ♀ 25
1	ns	s	s	s	a+s	a+s	a+s	a+s	a+s	a+s
2	ns	ns	a+s	a+s	a+3s	a+s+21s	a+2s+1s	a+3s	a+3s	a+3s
3	s	a+s	vls	vls	a+vls	a+vls	a+vls	a+vls	a+vls	a+vls
4	ns	ns	ns	ns	s	s	s	s	s	s
5	ns	ns	a+vls	a+vls	a+vls	a+vls	a+vls	a+vls	a+vls	a+vls
6	ns	ns	ns	ns	s	s	s	s	s	ls
7	ls	vls	vls	vls	vls	vls	a+vls	a+vls	a+vls	a+vls
8	ns	ns	ns	ns	sp	sp	s+sp	s+sp	s+sp	s+sp
9	ns	vls	vls	vls	vls	a+vls	a+s+vls	a+s+vls	a+s+vls	a+s+vls
10	ns	ns	ns	ns	s	s	s+ls	s	s+stsp	s
11	ns	ns	vls	vls	vls	vls	s+vls	s+vls	s+stsp	vls
12	ns	vls	sp	sp	a+s+sp	a+s+sp	a+s+sp	a+s+sp	a+s+sp	a+s+sp
13	ns	ns	ls	ls	ls	a+stsp	a+s+sp	vls	a+s+stsp	ls
14	ns	s	a+vls	a+vls	a+vls	a+s+vls	a+s+vls	a+vls	a+s+vls	a+vls
15	ns	ns	ls	ls	ls	s+ls	a+s+ls	vls	a+2s+stsp	ls
16	ns	ns	vls	vls	a+vls	a+s+vls	a+s+vls	a+vls	a+s+ls+stsp	a+vls
17	ns	ns	s	s	s	2s	a+s+sp	s	s+2sp	s
18	s	vls	vls	vls	vls	s+vls	a+s+sp	vls	2sp	vls
19	ls	ls	a+s	s	ls	a+s+ls	2sp	a+s	3sp	a+ls
20	ns	ls	s	s	s	s	sp	s	sp	s
21	vls	vls	vls	vls	vls	vls	s	vls	vls	vls
22	vls	s+vls	s+vls	s+vls	s+vls	s+ls	s+vls	s+ls	sp+vls	s+vls
23	s+vls	s+vls	s+vls	s+vls	s+vls	s+vls	s+vls	s+vls	s+vls	s+vls
24	2vls	2vls	2vls	2vls	2vls	2vls	2vls	2vls	2vls	2vls
25	a+2s+3vls	a+s+4vls	a+s+4vls	a+21s+3vls	a+21s+3vls	a+2ls+3vls	a+21s+3vls	a+21s+3vls	a+2ls+3vls	a+21s+3vls

The armature of the fused segments is interpreted by assuming that setae or aesthetae occupy positions on the antennule corresponding to those observed in subsequent instars. Segments 1, 2 and 3 constitute a single unit which resembles one segment. There are no setae on the first or second segment of this unit. The first seta arises from presumptive segment 3. Distal to this unit is another larger one consisting of segments 4 through 9 on which a large seta can be seen. The location of this seta is interpreted as being on future segment 7. Segments 10 through 18 form another elongate unit which has a seta near its distal end. This seta is accepted as indicating the position of segment 18 because of the presence of a very large seta on segment 18 of the CII antennule. The presence of a seta on the next complete distal segment is taken as evidence that this segment is number 19. This seta persists on segment 19 in the remaining instars. Following segment 19, the remaining segments are accounted for serially up to number 25.

Stage II. Each antennule is composed of 18 segments. The first segment bears a single seta and is separated by a suture. Distal to segment 1 is an elongate unit. This unit is believed to constitute segments 2 to 10. Two setae are present on this unit. The proximal seta arises from the 3rd presumptive segment and the distal one from the 7th presumptive segment (Fig. 2). This interpretation is based on the presence of a seta and an aestheta in the corresponding place on segment 3 and a very long seta on segment 7 in CIII. Distal to this unit is a small unit which constitutes segments 11 and 12. Distal to segment 12, the remaining segments are distinctly separated from each other and can be counted easily.

Stage III. The antennule possesses 23 apparent segments. The apparent 3rd segment bears a long seta and is fused with the 4th segment (Fig. 3). The point of emergence of this seta has been interpreted as marking off the distal margin of future segment 3. This interpretation is based on the presence of a very long seta on segment 3 in both sexes of CIV. The remaining segments are distinct (Figs. 3, 4, 5). Slight differences in armature occur between left and right antennules of this stage, but it was not determined if these differences were related to sex.

Stage IV. The adult condition of 25 distinct segments is assumed in this stage (Figs. 6, 7). The right antennule of the female and the left antennule of the male have the same armature on segments 1 to 12, but they have minor differences in some of the succeeding segments.

Stage V. Spines appear on segments 17 to 20 of the right antennule of the male (Fig. 8, 9). The left antennule of the male is similar to the left antennule of the female CVI stage.

Adult Male. The right antennule of the male is geniculate between segments 18 and 19 (Figs. 10, 11, 12). The left antennule extends beyond the tips of the caudal rami.

Plate I. Structure of the antennule. Figure 1, CI. Figure 2, CII. Figure 3, segments 1-15 of CIII. Figure 4, segments 16-21 of CIII. Figure 5, segments 22-25 of CIII. Figure 6, segments 1-18 of CIV. Figure 7, segments 19-25 of CIV. Figure 8, segments 1-16 of CV. Figure 9, segments 17-25 of CV. Figure 10, segments 1-13 of the right adult ♂. Figure 11, segments 14-21 of right adult ♂. Figure 12, segments 22-25 of right adult ♂. Figure 3, 4, and 5 drawn to twice the scale indicated on the plate.

ANTENNA

The antennae of the copepodid stages consist of 2 basal segments, the coxa and basis, an endopod and an exopod (Fig. 13). The endopod is two-segmented whereas the exopod is nine-segmented. The coxa has one seta throughout all copepodid stages. The basis has 1 seta in CI and 2 in all succeeding stages. The exopod has 12 setae in all stages. The basal segment of the endopod has 2 setae. The terminal segment has 11 setae in CI, CII, CIII, 14 setae in CIV and 15 setae in CV and CVI. The sutures are poorly developed in CI. In CII the exopod is completely segmented and the endopod shows better development of the suture between proximal and distal segments. The only further development is that sutures and armature become more distinct.

MANDIBLE

The mandible consists of a coxa, a basis with 4 setae, a four-segmented exopod with 6 setae, and a two-segmented endopod (Fig. 14). The proximal segment of the endopod has 4 setae. The distal segment has 6 setae in CI to CV and 8 in CVI.

The masticatory process has 11 teeth in CI; this number is reduced to 8 in all succeeding stages. Setae are absent, but a small process is present.

MAXILLULA (TABLE II)

The maxillulae attain in CI the general form which they maintain throughout the remainder of development. In CII the maxillulae are more elongated and additional setae occur on the epipod of the coxa, on the gnathobase of precoxa, and on the endopod. There is no further change in the armature of this appendage except for some additional setae on the precoxa and the epipod of the coxa. Generally, the setae become stouter and the maxillulae become enlarged.

TABLE 2. Armature of the maxillulae. Entries refer to the number of setae. The letter h, hairs.

Copepodid Stages	I	II	III	IV ♀	V ♀	VI ♂
Precoxa						
Medial	2	3	3	4	[illegible]	4
Anterior	7	7	8	9	9	10
Coxa						
Epipod	4	6	8	9	9	9
Lacinia	4	4	4	4	4	[illegible]
Basis						
Posterior	1	1	1	1	1	1
Lacinia	4	4	4	4	4	4
Exopod, distal	6	6	6	6	6	6
Endopod, total	10+h	12	13	13	13	13

MAXILLA (TABLE III)

The maxilla in CI is so small that counting the number of endites is difficult. Dissection of the appendage could result in breaking some of the setae. The number of endites in this stage is approximately 18. The total number of endites is increased to 23 in CII. In CIII the appendage attains its definitive form. The total number of endites reaches 24.

TABLE 3. Armature of maxillae. Entries refer to the number of setae.

Copepodid Stages	I	II	III	IV ♀	V ♀	VI ♂
Endite 1	3	4	5	5	5	5
Endite 2	3	3	3	3	3	3
Endite 3	3	3	3	3	3	3
Endite 4	3	3	3	3	3	3
Endite 5	2	4	4	4	4	4
Remainder	4	6	6	6	6	6
Total Setae	18	23	24	24	24	24

MAXILLIPED (TABLE IV)

Stage I. The maxilliped has the form of an elongate appendage with several lobes. Segment 1 is not distinct and is devoid of setae. There are 3 lobes on segment 2. The first lobe has no setae, but there is 1 seta on the second lobe, and the third lobe has 2 setae and also possesses teeth. The unsegmented endopod bears 5 setae.

TABLE 4. Armature of maxilliped. Entries refer to the number of setae. Abbreviations: t, teeth present; c, comb like teeth; h, hairs; un, unsegmented.

Copepodid Stages	I	II	III	IV ♀	V ♀	VI ♂
Sympod	0	0	1	1	1	1
Segment I						
Segment II						
Lobe 1	0	1	2	2	2	2
Lobe 2	1	2	3	3	3	3
Lobe 3	2+t	2+t	3+t	3+t	3+t	3+t+h
Segment III	1+t	2+t	3+t	3+t	3+c	3+c
Endopod	un	un	un			
Segment 1				2	2	2
Segment 2				3	3	3
Segment 3				2	2	2
Segment 4				2	2	2
Segment 5				2	2	2
Segment 6				4	4	4
Total Endopod	5	9	9	15	15	15

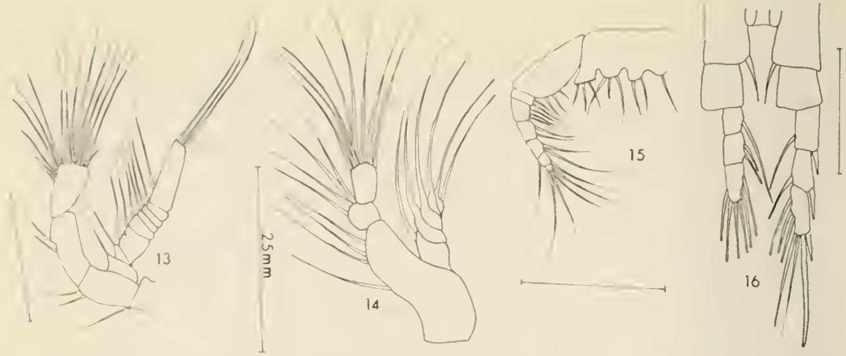

Plate II. Figure 13, structure of the antenna of the adult female. Figure 14, structure of the mandible of the adult male. Figure 15, structure of the maxilliped of the adult male. Figure 16, structure of the third leg of the adult male. The exopod of the right leg and the endopod of the left leg were removed. All scale lines=0.25 mm.

Stage II. The individual segments of the endopod are not distinguishable because of weak sutures. The number of setae on the endopod is increased to 9.

Stage III. Segmentation of the sympod becomes pronounced in this stage. Segment 1 of the sympod bears a single seta which is apparent in the rest of the instars.

In CIV the maxilliped attains its definitive form, with a full complement of setae as well as segments (Fig. 15).

First Swimming Leg (Table V)

In CI, the leg consists of a coxa and a basis without armature, together with an exopod and endopod, each consisting of a single segment with armature. The armature of the endopod does not change in succeeding stages, although it is divided into 2 segments in CIV and thereafter.

The exopod becomes two-segmented in CII and three-segmented in CV. The spines on the CI exopod are considered homologous to those of the terminal segment of the CV exopod (Comita and Tommerdahl, 1960). A seta is added to the medial side of the first segment in CIII and to the medial side of the second segment in CV.

A seta appears on the medial surface of the coxa in CII and hairs develop on the coxa in CVI. The definitive form of the leg is attained in CV although the appendage becomes stouter and enlarged in CVI.

Second Swimming Leg (Table VI)

The coxa and basis are devoid of armature throughout development, except for a single seta which occurs on the medial side of the coxa in CII and all subsequent stages. The endopod and exopod follow essentially the same

Table 5. Armature of the first swimming leg. Entries refer to the number of segments or setae. Abbreviations: sp, spine; h, hairs. The armature designated with * is for the single whole branch although the numbers are entered in the table to indicate the two segments are present in that stage.

Copepodid Stages	I	II	III	IV ♀	V ♀	VI ♂
Coxa	0	1	1	1	1	1+h
Exopod, no. of segments	1*	2	2	2	3	3
Segment 1						
Medial	0	0	1	1	1	1
Lateral	sp	sp	sp	sp	sp	sp
Segment 2						
Medial	no segment				1	1
Lateral					0	h
Segment 3						
Medial	1	1	2	2	2	2
Lateral	sp	sp	sp	sp	sp	sp+h
Terminal	3	3	3	3	3	3
Endopod, no. of segments	1*	1*	1*	2	2	2
Segment 1						
Medial	1	1	1	1	1	1
Segment 2						
Medial	2	2	2	2	2	2
Lateral	1	1	1	1	1	1
Terminal	3	3	3	3	3	3

pattern of development. In CI each consists of a single segment which lacks the armature of the definitive segment 2. Both become two-segmented in CII and three-segmented in CV. A seta appears on the definitive second segment of the exopod in CII and of the endopod in CIII. A second seta is added to the CIV endopod. The armature of the terminal segment of each ramus remains unchanged throughout development except for the addition of a seta to the medial surface in CII. The terminal seta of the exopod becomes serrated on the outer edge in CV and CVI.

Third Swimming Leg (Table VII)

In CII, the endopod and exopod are single-segmented. Setae are present on both rami, and spines are present on the exopod. Both rami become two-segmented in CIII and an additional seta occurs on the medial side of the definitive third segment of the exopod. A spine and seta are added to the exopod and a seta to the endopod in CIV. The leg reaches definitive form in CV. The terminal seta of the exopod is stout and serrated along its length (Fig. 16).

Table 6. Armature of the second swimming legs. Entries indicate number of setae or segments. Abbreviations: ns, no segment; h, hairs; sp, spine. The armature designated with * indicates that entire branch is a single segment; ** indicates that segment 1 is separated from the rest of the appendage by a suture; the remainder is a single segment which is composed of future segments 2 and 3.

Copepodid Stages	I	II	III	IV♀	V♂	VI♂
Coxa						
Medial	0	1	1	1	1	1
Basis	0	0	0	0	0	0
Exopod, no. of segments	1*	2**	2**	2**	3	3
Segment 1						
Medial	0	h	1	1	1	1
Lateral	sp	sp	sp	sp	sp	sp
Segment 2						
Medial	ns	1	1	1	1	1
Lateral	ns	0	sp	sp	sp	sp
Segment 3						
Medial	1	2	2	2	3	3
Lateral	sp	sp	sp	sp	sp	sp
Terminal	3	3	3	3	3	3
Endopod, no. of segments	1*	2**	2**	2**	3	3
Segment 1						
Medial	1	1	1	1	1	1
Segment 2 (Medial)	ns	ns	1	2	2	2
Segment 3						
Medial	1	2	2	2	2	2
Lateral	1	1	1	1	1	1
Terminal	3	3	3	3	3	3

Fourth Swimming Leg (Table VIII)

The fourth leg occurs as a bilobed appendage in CII. Each lobe has 2 setae.

The exopod and endopod are single-segmented in CIII, two-segmented in CIV and three-segmented in CV. The full armature and segmentation are developed in CV. In CVI the lateral spine on the terminal segment of the exopod is coarsely serrated; in general, the whole appendage is more elongated than in previous stages.

Fifth Swimming Leg (Table IX)

These appendages in all stages are symmetrical in the female and asymmetrical in the male. In CIV, the exopod and endopod of both sexes are com-

TABLE 7. Armature of the third swimming legs. Entries indicates the number of segments or setae. Abbreviations: h, hairs; c, comb; sp, spine. The * indicates that the entire branch is a single segment; ** indicates that segment 1 is separated from the rest of the appendage by a suture and the remainder is a single segment which is composed of future segments 2 and 3.

Copepodid Stages	II	III	IV ♀	V ♂	VI ♂
Coxa					
Medial	h	1	1	1	1
Lateral	h	0	0	0	0
Basis (lateral)	0	1	1	0	0
Exopod, no. of segments	1*	2	3	3	3
Segment 1					
Medial	0	1	1	1	1
Lateral	sp	sp	sp	sp	sp
Segment 2					
Medial	--	--	1**	1	1
Lateral	--	--	sp	sp	sp
Segment 3					
Medial	1	2	2	3	3
Lateral	sp	sp	sp	sp	sp
Terminal	3	3	3	3	3
Endopod, no. of segments	1*	2	2	3	3
Segment 1 (Medial)	1	1	1	1	1
Segment 2 (Medial)	--	1*	1**	2	2
Segment 3					
Medial	1	1	2	2	2
Lateral	1	1	1	1	1
Terminal	3	3	3	3	3

posed of single segments. The right exopod of the male is slightly larger and has a larger terminal outer spine than the left exopod (Fig. 17). The male right endopod is smaller than the left and has 2 terminal setae. The inner terminal seta of the female exopod is larger than the outer (Fig. 18); the endopod has 2 terminal setae.

All exopods become two-segmented in CV. In the female, the distal end of the terminal segment of the exopod bears 4 spinous processes, of which the inner is the stoutest (Fig. 19). In the male, the terminal segment of the right exopod is much larger than the terminal segment of the left exopod (Fig. 20). Two spines occur on the terminal segment of the left exopod, only one spine occurs in the comparable position in the right exopod and this spine is large and stout.

TABLE 8. Armature of fourth swimming leg. Entries refer to the number of setae or segments. Abbreviations: s, serrated; h, hair; sp, spine. The armature listed under 1* is for the single whole branch; ** indicate that segment 1 is separated from the rest of the appendage by a suture, remainder is a single segment which is composed of future segments 2 and 3.

Copepodid Stages	III	IV ♀	V ♂	VI ♀
Coxa (Medial)	1	1	1	1
Basis	0	0	0	0
Exopod, no. of segments	1*	2	3	3
Segment 1				
Medial	0	0	1	1
Lateral	sp	sp	sp	sp
Segment 2				
Medial		1*	1	1
Lateral		sp	sp	sp
Segment 3				
Medial	1	2	3	3
Lateral	sp	sp	sp	sp
Terminal	3	3	3	3
Endopod, no. of segments	1*	2	3	3
Segment 1 (Medial)		1	1	1
Segment 2 (Medial)		1*	2	2
Segment 3				
Medial	1	1	2	2
Lateral	1	1	1	1
Terminal	3	3	3	3

The endopods remain one-segmented in CV and in CVI. In the CV female, two setae occur. The endopods are asymmetrical in the CV male and bear two setae plus hairs.

Stage VI. Female: The terminal segment of the exopod is triangular in shape and pointed at the distal end. It has 1 seta and 2 spines on its lateral side. Along the medial edge is a bladelike margin which is divided into fine teeth (Fig. 21). The endopod is one-segmented and bears 2 setose structures at its distal end. The coxa bears a sensory seta and the basis has a lateral spine near its distal end.

Stage VI. Male: The fifth legs in the adult male are distinctly asymmetrical (Fig. 22). The coxa of the right leg bears a sensory spine. The basis of the right leg possesses two stout spines on the medial side, a small spine on the lateral side, and a distally placed stout hook that reaches nearly to the end of the first exopod segment. The basis of the left leg possesses one lateral

Table 9. Armature of the fifth legs. Entries refer to the number of setae, or abbreviations: o, no armature; h, hairs; sp, spine; b sp, blunt spine; stsp, stout spine; setae: b, blade like.

Copepodid Stages	IV ♂	IV ♀	V ♂	V ♀	VI ♂		VI ♀
					L	R	
Coxa, Posterior	0	0	0	0	..	sp	sp
Basis							
Medial	0	0	0	0	0	2sp	0
Lateral	h	h	h	h	sp	sp	sp
Endopod, no. of segments	1	I	I	1	1	I	1
Terminal	2+h	2+h	2+h	2+h	3sp	h	2+h
Exopod, no. of segments	I	1	2	2	2	2	2
Distal segment							
Medial	0	0	0	0	0	0	h
Lateral	0	0	sp	0	0	stsp	1+2sp
Terminal	2sp	2sp	stsp	4sp	2sp	sthk	b

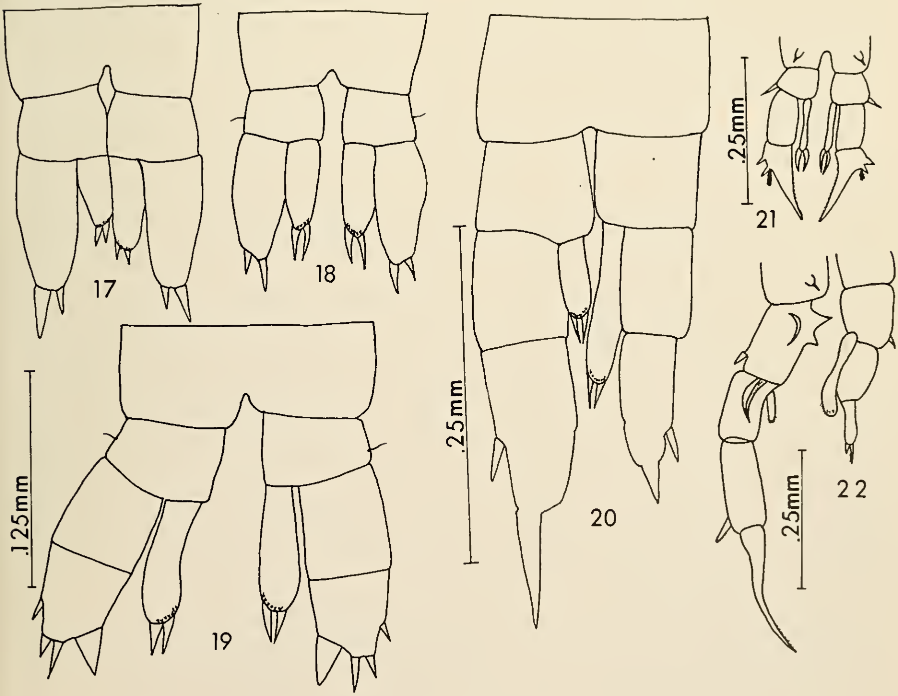

Plate III. Structure of the fifth leg. Figure 17, CIV ♂. Figure 18, CIV ♀. Figure 19, CV ♀. Figure 20, CV ♂. Figure 21, adult ♀. Figure 22, adult ♂.

spine. The endopod of the left fifth leg is an elongated structure and bears 3 small subterminal spines. The endopod of the right fifth leg is small and bears a number of very small spines. The left fifth exopod bears a terminal setose structure in addition to a spine. The setose structure appears to be modified from one of the spines present in CV. The right exopod is elongated and bears a large sickle-shaped terminal spine.

DISCUSSION

The genus *Diaptomus* has a world-wide distribution in fresh and saline waters; although there are 78 recognized species in North America, rarely do congeneric copepods occur together in great numbers (Pennack, 1957; Cole, 1961). As Cole pointed out, most of these congeneric occurrences consisted of species referable to different subgenera. The genus *Diaptomus* is subdivided into 14 subgenera (Wilson, 1959); many of these subgenera are recognized as genera by European workers. Thus the frequency of congeneric occurrence is biased by the taxonomic system in use. However, of more importance than precise enumeration of congeneric occurrence or the validity of one genus versus many genera approaches to the taxonomy of these organisms, are the problems of the ecological requirements and of the mechanisms of speciation of this complex of species.

Diaptomus siciloides occurred with *D. clavipes* in the local ponds from which these crustaceans were collected. Although these two species are relegated to different subgenera, they have striking similarities in their adult and larval structures but differ markedly in size and in the structure of the antennule and fifth legs, e.g., the armature of the right antennule of the males differs considerably in segments 8, 15, 16, 17, 18, 22 and 23. By contrast, the left antennule of female *siciloides* and *clavipes* is similar except for size and number of setae on segment 2. The armature of antennae, mandibles, maxillulae, maxillae and maxillipeds, and legs 1 to 4 is similar in the two species.

Although these two species frequently occur in the same body of water, they may occupy different niches (Pennak, 1957). Hutchinson (1951) has suggested that size-differences between species of copepods in the same genus may reduce competition because of different food selection. For example, the digestive tract of *Arctodiaptomus laticeps* (1.54 to 1.65 mm long) contained *Melosira* but that of *Eudiaptomus gracilis* (1.14 to 1.23 mm long) contained minute algae and tripton (Fryer, 1954).

Little work has been done on physiological differences at the species level in copepods. However, differences in size, breeding cycle and food preference indicate the presence of physiological differences. These probable physiological differences are reinforced by morphological differences. If *D. clavipes* and *D. siciloides* are typical for the genus, speciation at the morphological level primarily involved divergence in size and structure of the male antennule and the fifth legs. These differences are evident in *Eudiaptomus vulgaris* Schmeil and *Mixodiaptomus laciniatus* Lill. (Ravera, 1953). The fifth legs of CIV males and females differ only slightly with their counterparts of CIV *D. clavipes* and *D. siciloides*, even though each species is in a separate subgenus (or genus?). The structures of the various CV fifth legs show more divergence. In the females, the exopod of *D. clavipes* bears one large, thick spine and three smaller spines of similar size. The three smaller spines are more variant in size in *M. laciniatus* and only two spines are clearly evident in *E. vulgaris.* Among the males there are slight differences in the length of the endopods and in the size and number of terminal spines on the endopods. The exopods are more nearly alike. The structural differences among the fifth legs are accentuated further in the adults of both sexes and the legs become species distinctive. These structural differences may serve to reduce or prevent interbreeding among species of *Diaptomus* whose physiological and ecological requirements permit some degree of sympatry.

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THE UNIVERSITY OF KANSAS
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A STUDY OF THE BIOLOGY OF TWO SPECIES OF PODOCINIDAE (ACARINA: MESOSTIGMATA)

By

Calvin L. Wong

Vol. XLVII Pages 575-600 October 11, 1967 No. 8

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THE UNIVERSITY OF KANSAS
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A Study of the Biology of Two Species of Podocinidae (Acarina: Mesostigmata)[1]

By

Calvin L. Wong

ABSTRACT

Two predatory mites of widespread distribution, *Podocinum pacificum* and *P. sagax* (Mesostigmata), were raised in laboratory cultures. The podocinids were fed on Collembola and acarid mites. Culture methods are detailed. While *P. pacificum* reproduces parthenogenically, *P. sagax* is shown to require mating in order to oviposit. In addition, repeated mating is necessary for continued reproduction. Life history, feeding, and ecdysial behavior also are reported. The low survivorship of *P. sagax* males is suggested as a primary limiting factor on the geographic distribution of the species. The mites used in the study were collected in Kansas and Florida.

INTRODUCTION

Since the description of *Podocinum sagax* by Berlese in 1882, and the erection of the family Podocinidae by the same author in 1913, this group has expanded until it now comprises 15 species in two genera. Members of the family are nearly cosmopolitan in distribution through the tropics, *P. pacificum* having been recorded from every continent except Australia and Antarctica. Further collecting will undoubtedly reveal new locality records for these mites. This study reports *Podocinum* from five states as well as new records of Panama.

The almost total absence of biological investigations on members of this family of active forest litter predators belies the possible ecological importance of such a widespread group. The effect of predators on leaf and log litter decomposers, as well as on small, fungus feeding animals, must be under-

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stood before the dynamics of forest micro-communities can be properly evaluated. This study considers some of the fundamental biological characteristics of *Podocinum pacificum* and *P. sagax* which must be known in order to appreciate the ecological role of these species.

Studies of the biology of the podocinids may also be expanded eventually to test some of the present systematic and genetic concepts concerning the Mesostigmata. *Podocinum pacificum* and *P. sagax* would be especially useful tools in such problems since their ranges and habitats overlap, at least in some areas, and because both mating and parthenogenic forms of reproduction occur in this genus; *P. pacificum* is known only from females, which have been shown in this study to be capable of producing fertile eggs, while *P. sagax* apparently requires mating in order to oviposit.

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SYSTEMATIC RELATIONSHIPS

Podocinum sagax was originally described by Berlese in 1882, in the genus *Laelaps*. In the same year, he erected the genus *Podocinum* for this species and described a second species, *P. pacificum*, in 1896. Seventeen years later, he added three new species to the genus and created the tribe Podocinini (1913a).

No further changes were made until Evans and Hyatt reviewed the group in 1958 and described eight new species, erecting the genus *Podocinella* for three of the 13 species. In the same paper, the tribe Podocinini was raised to familial status. The latest additions to the Podocinidae are DeLeon's *Podocinum catenulum* from Tennessee and the monotypic *P. pugnorum* from Florida.

Baker and Wharton (1952) had placed *Podocinum* in the rather heterogeneous subfamily Podocininae of the family Phytoseiidae. Evans and Hyatt (1958) consider the Podocinidae to be most closely related to the Phytoseiidae, with affinities to the Ascidae and Epicriidae, but distinctive enough to warrant separation from these families. Camin (1962, personal communication) expressed opinions similar to those of Evans and Hyatt.

MATERIALS AND METHODS

The majority of specimens used in this study were collected in log and leaf litter which was brought into the laboratory in plastic bags and processed with Berlese funnels. Litter stored in these bags, when protected from desiccation by the tightness of the seal or by the occasional addition of distilled water, has yielded *Podocinum* as long as 60 days after the original collection. Individual mites were always handled in the laboratory with a small (size 0-3), moist, sable water-color brush.

Two types of rearing chambers were used: Small, clear plastic boxes, and small stender dishes. The boxes are square in shape, about 20 mm on a side and 15 mm deep, while the round stenders have a 30 mm inside diameter and are also 15 mm deep. A dry mixtures of plaster-of-paris and activated carbon, in the ratio of 500 to 60 by weight, respectively, was combined with water and added to the chambers to about one-third of the depth. When the substrate had hardened, the excess carbon on the surface was washed off in water as otherwise it adheres to the tarsi of the mites. The plastic boxes were found to be better suited for individual and small group cultures, since they are more convenient to handle and store and single mites are more easily located in the smaller area.

Fluctuations of relative humidity in the chambers were reduced by the regular addition of distilled water to the substrate. Care was taken to avoid excess water, however, since condensation droplets in the stender dishes could trap and kill the eggs or early postembryonic stages. This accumulation of free water did not occur in the plastic rearing chambers. The high humidity was also found to encourage the growth of mold which occasionally attacked and killed the *Podocinum* eggs, as well as destroying quantities of the Collembola eggs being reared as prey. A 5% aqueous sodium benzoate solution, initially added in sufficient quantity to moisten the dry substrate, was useful in inhibiting the growth of mold.

The rearing chambers were also provided with several small shreds of fiberglass filter paper for oviposition sites. Eggs laid on these pieces of filter "paper" were probably less exposed to predation by acarids or podocinids than eggs laid on the substrate. The fiberglass is preferable to conventional filter paper because the former material does not provide an organic substance on which mold can grow. If required, eggs laid on the filter paper could be easily moved to another container with the fiberglass shred. The eggs were rarely handled individually, and, if they were found attached to the walls of the chamber, they were left in place to hatch. If an egg was laid directly on the substrate, it could be removed by excavating a piece of the plaster-carbon material with the firmly attached egg.

The cultures were normally examined about twice daily to determine whether or not ecdysis had occurred, to record oviposition, and to add food or water, if necessary. It was found early in this study that recently molted mites exhibited no physical or behavioral differences from the previous stage which were visible under the dissecting microscope. Although molted larvae were readily distinguished by the addition of a fourth pair of legs, there was no apparent change in size or sclerotization shortly after a post-larval ecdysis. Also, due to the short duration of the ecdysial process, this was rarely observed. It was found that all the post-larval stages could be marked with a 10 percent india ink solution, with little danger of harm. The ink used, with good results, was Higgin's "Engrossing Black," which was applied with a fine, camel's-hair brush from which most of the bristles had been removed. An excess of ink occasionally resulted in the death of a protonymph. It became possible to use distinctive markings on individual mites when a number of mites, of approximately the same size, were present in a single chamber. "Dorsal maps" of the various mites were sketched along with the daily observation notes, so that when ecdysis occurred, the presence of a mite without an ink marking would be immediately evident.

Two types of prey were used primarily in the course of this study. One was an acarid mite, *Tyrophagus* sp., and the other was a collembolan of the family Poduridae, *Neobeckerella* sp. Both were collected in the vicinity of Lawrence, Kansas, and cultured in the laboratory specifically for use as prey in this investigation. While the acarids were easily raised in small stender dishes, larger dishes, with an inside diameter of about 40 or 50 mm were found to be better suited for the more active podurids. The substrate for these rearing pens was the same as that used for the podocrinids, and dry "active yeast" grains were used as food. Yeast was also placed in the podocinid chambers when the prey were introduced since starved acarids were likely to attack and kill any of the stages of the podocinids, available food normally prevented this. Reproduction of the podurids in the podocinid culture chambers usually did not reach a useful level. In contrast, it was frequently necessary to remove most of the adult acarids to prevent overwhelmingly large populations since the large nymphs and adults of the acarids were apparently not subject to predation by the podocinids.

A study of the life history and reproductive rate of *Tyrophagus* showed a short developmental period and a high reproductive potential. The egg to adult period for 15 individuals took about 17-18 days, distributed as follows: egg stage, 6-8 days; larva, 3-4 days; larval pre-molt, 1-2 days; protonymphal stage, 2-3 days; first nymphal pre-molt, 1 day; tritonymphal stage, 1 day;

final pre-molt, 1-2 days. The adult pre-oviposition period was 1-2 days. The reproductive rate, for several mature females, over a period of 34.9 female-days, was an average of 19.1 eggs per day.

While the acarids used in this study were easily transferred with a camel's-hair-brush, or a micro-dissecting needle, the podurids were difficult to handle due to their ability to jump, as well as their susceptibility to damage when handled with a water-brush. In order to effect the transfer of the active stages of the podurids, a microaspirator was constructed from a thin-walled, glass tube, with an outside diameter of 5 mm. This was drawn out over a bunsen flame and cut, leaving a tapered portion approximately 10 mm long and about 1 mm in diameter at the orifice. The total length of the glass tube was about 50 mm long. A small wad of cotton was loosely packed into the tube at the wide end of the tapered section. This served as the filter to prevent inhalation of the insects. The wide end of the glass tube was also covered with a small piece of fine, silk bolting cloth before this end was tightly fitted into a 400 mm length of rubber hose which served as a sucking tube. Using this type of aspirator, individual or large groups of podurids could be rapidly and safely transferred.

For microscopical examination, the mites were either placed alive into the clearing solution or killed and preserved in 95% ethyl alcohol before clearing. They were cleared in Nesbitt's solution and mounted in Hoyer's modification of Berlese's medium.

The location of specimens is coded as follows: (1) British Museum; (2) U.S. National Museum; (3) Snow Museum, The University of Kansas; (4) Institute of Acarology, Agricultural Experiment Station, University of Ohio, Wooster, Ohio; (5) Dept. of Agriculture, University of Missouri; (6) Donald DeLeon, Erwin, Tennessee.

Podocinum pacificum

Podocinum pacificum Berlese, A. 1896. Atti. Soc. Veneto (2), *2*, pt. 2: 319.

The locality records include the following: Eurasia. *Italy:* Florence and Naples. Berlese (1913a) reports this species to be common under stones, among ant nests and humus; *Austria:* Leopoldsberg, Vienna Woods, 4 July 1948, W. Kühnelt, oak litter (1); *Sikkim:* 8,610′, Lachung, 18 Feb. 1952, T. Clay, moss on rotting log (1).

Africa. *Algeria:* Maison-Carrée, 20 Dec. 1956, 20 Nov. 1957, among mushrooms; Boufarik, 28 Jan. 1958, soil from a field of *Lippia*; Benni-Messous, 20 Oct. 1957, litter at base of *Ulmus*.

South America. *Argentina: Tucumán;* 800 meters, Quebrada de la Higurea, Choromoro, 15 March 1953, P. Wygodzynsky, fallen leaves and decaying wood (1); 800 meters, Parque Aconquija, Jan.-March 1953, P. Wygodzynsky, rotting leaves (1); Quebrada de la Angostura, Tafi, 17 Feb. 1953, P. Wygodzynsky, rotting forest litter (1).

North America. *Mexico:* Puebla; 6 mi. E. Teziutlán, 22 July 1955, R. E. Beer, leaf mold (3); Veracruz; Cuesta de Acultzingo, 16 Jan. 1942, F. Bonet, from dead leaves (2); United States: *California:* Alameda Co., Berkeley, 29 Oct. 1951, W. C. Bentinck (1); University of California Botanical Gardens, 29 Oct. 1949, R. E. Beer, leaf mold (3); *Kansas:* Douglas Co., Lawrence, 3 July 1946, P. W. Jameson Jr., from nest of *Microtus ochragaster* (2), 8 April 1952, R. E. Beer, under rock (3), 7 Feb., 2 April 1952, Nov. 1961, A. B. Amerson & P. A. Thomas, Neotoma nest (3); Kans. Univ. Nat. Hist. Res., May 1958, J. W. Kliewer, Nov. 1959, R. E. Beer, Jan., Feb., April, Oct., Nov. 1960, Jan., April 1961, R. E. Beer & C. L. Wong, May 1962, M. A.

Hoy, leaf litter from mesophytic oak-elm woods on shallow loamy soil among limestone outcroppings (3); Franklin Co., near Ottawa, 20 May 1954, R. E. Beer, in moss (3); Johnson Co., 18 April 1950, D. T. Daily, cornfield litter (3): Cherokee Co., 4 mi. S., 1-½ E. of Galena, 8 April 1955, R. E. Beer, under rock in dense woods (3); *Missouri:* Lawrence Co., Verona, 4 May 1958 (5); Boone Co., Columbia, 22 Feb. 1954, 11 July 1959, 26 March 1961 (5); *Illinois:* Champaign Co., Urbana, 10 Jan. 1939, P. C. Stone, in nest of *Microtus ochragaster* (2); *Arkansas:* Washington Co., Fayetteville, 13 April 1956, D. S. Lang, under rocks (3); Yell Co., 2 mi. S. Rover, 6 April 1961, N. MacFarland, leaf litter (3); *Tennessee:* Unicoi Co., Erwin, 1961-1963, D. DeLeon, from litter and decaying apples on ground (6); *Virginia:* Fairfax Co., Mt. Vernon, 10 Dec. 1944, Andre, in moss (2); Albemarle Co., Charlottesville, 15 Feb. 1948, R. L. Hoffman, in leaf mold (2); *North Carolina:* Polk Co., Tryon, July 1937, D. C. Peattie, on wild ginger flower (2); *Louisiana:* Madison Co., Tallulah, 10 June 1959 (3); *Mississippi:* Marshall Co., Wall Doxy State Park, 13 April 1962, C. L. Wong, under log at edge of oak, elm, redbud hardwood forest (3); *Florida:* Levy Co., Manatee Springs State Park, 12 April 1962, C. L. Wong, in rotting log on leaf litter in oak, sweetgum hardwood forest on shallow sandy soil over limestone (3); Highlands Co., Highlands Hammock State Park, 5, 8, 10 April 1962, C. L. Wong, in rotting hardwood logs and leaf litter in wet oak-palm woods on shallow loamy soil over moist sand (3); Marion Co., Moss Bluff, 22 May 1958, H. L. Greene & M. H. Muma, pine and hardwood leaf litter (in Citrus Exp. Sta. Coll., Lake Alfred, Fla.): *Maryland:* Prince Georges Co., Laurel, Patuxent Research Refuge (4); "New England" (4); *"North Carolina"* (4).

BIOLOGICAL DESCRIPTION

1. *General Notes*

Podocinum pacificum is a medium size mite, bearing a pair of highly elongate front anterior legs which are modified as tactile sensory structures. These are characteristic of the family. Adults are moderately sclerotized and in life are 415-460 μ long, exclusive of the gnathosoma, and 108-142 μ wide. The anterior legs (about 1040-1355 μ long when extended) bear a pair of long, whiplike, sensory setae apically. Using a dissecting microscope and strong illumination at 80× magnification, the reticulated pattern of the dorsum can be distinguished.

When active the adults and deutonymphs walk moderately rapidly, holding the anterior pair of legs looped dorsally over the propodosoma, the tibiae and tarsi pointed forward and downward while sweeping transversely and in unison across the substrate in front. The looped portions of these sensory structures are thus in a position to detect stimuli from an anterodorsal as well as a directly anterior direction. This sensitivity is readily demonstrable when a moist or inked water brush is brought near the mite. When thus stimulated, the usual response of the mite is to run backwards rapidly.

If the approach of the brush is not abated after a short time, the response changes, and the mite flattens itself against the substrate with the sensory legs folded flat over the body. If touched while in this attitude, the response is again a rapid retreat. Reaction to the approach of a dry brush is considerably less pronounced, which is interpreted as an indication that a chemosensory response to the moist brush is involved. If the podocinid is walking and encounters a large acarid or podurid, stimulation of the long sensory setae is likely to effect the same backward running response. Since the setae are normally in a position to encounter any object slightly more than one body length from the mite, and since the angle through which the legs sweep is normally close to 90 degrees, this means of detecting potential danger is rather effective. It is likely that some discrimination of the size of objects encountered is also possible. The terminal segment of the legs, bearing the apical pair of sensory setae, is held in a position such that one seta is normally carried above the other. Therefore, while the lower seta is apparently in contact with the substrate, the upper one is in a position to respond to objects as tall or taller than the mite. Certainly, a differential response to different sized acarids or podurids was frequently observed.

When one podocrinid encounters another, contact is usually made with the long setae of the first legs, and the result is a slight mutual inhibition of movement. This inhibition is generally more apparent if the two mites involved are both adults and results in mutual stroking of the anterior legs. Attempts to evoke a similar response by stroking the legs with a hair from a water brush results in an avoidance reaction.

The rate of activity seems to increase with an increase in light, this apparently being a kinetic reaction as a skototaxis has not been detected.

A similar response has been observed when a excess of water applied to the substrate results in the presence of free water on the surface. When the mite encounters a relatively dry area, activity is reduced to a normal level. Occasionally, mites become trapped in water droplets and die when apparently unable to extricate themselves. The immature stages are particularly prone to this danger, and when a culture chamber is permitted to become crowded, increasing numbers of mites may be found trapped in condensation drops on the underside of the stender lids. Under these conditions, only a slight amount of moisture may prove fatal since the accumulation of waste products from both acarids and podocinids greatly increases the glue-like quality of the droplets.

Among the other causes of death to the mites in culture are desiccation, predation by acarids, fungal attack, starvation and cannibalism. The last two causes will be discussed in the section on feeding.

Death from desiccation usually occurred only in chambers with insufficiently thick substrate layers which did not permit the retention of enough water for an adequate reserve. In such chambers, it is possible that predation by acarids is increased, since desiccation also affects the normally moist yeast, making it less attractive as a food. In any case, acarids were seen to feed on dead podocinids although dehydrated yeast was available, while under normal circumstances, the yeast was preferred to dead podocinids. In moist chambers without adequate yeast, large acarids frequently attacked live podocinids.

Fungal attack on podocinids is not common, although several kinds of fungi occur regularly in the rearing chambers, especially on the yeast. Occasionally, a fungus will appear in an opened, though otherwise normal-looking, dead mite. This particular growth has not been observed in living individuals; however, it usually occurs in mites whose death would be unexplained othewise. These mites show varying degrees of separation along the posterior conjunctival membrane between the dorsal and ventri-anal shields due to the growth of the fungus in the opisthosoma. In some cases, the fungus has resulted in an extreme reflection of the dorsal surface as it has neatly "dissected" off the dorsum anteriorly as far as the gnathosoma. The spore-forming stage of this fungus has not been recovered and the fungus has not been identified.

Natural death or death due to physiological limitations is difficult to evaluate in such a study where many other factors may be responsible. However, cases of progressive reduction in oviposition rate and eventual cessation of egg-laying before death are considered to include natural deaths.

Podocinum pacificum is susceptible to death by cold. Although adults have been berlesed from leaf litter collected during the winter, attempts to store any and all stages in a refrigerator at 5-10° C have been unsuccessful. In one case, the cultures were placed directly into the refrigerator, and in the second attempt acclimatization was used, the chambers receiving two cold treatments each day for one week. These treatments were initially of a half hour duration, increasing by half-hour units until the exposure period was two hours, whereupon the increment was raised to one hour per day. After one week, the chambers were left refrigerated for two months at the end of which time all stages were dead. These experiments included over 20 mites of each stage, except the egg and larval stages which involved about ten individuals each. It was found, however, that both podurids and acarids in the egg stage survived the treatment.

2. *Feeding Behavior*

While *Podocinum pacificum* could be raised from egg to adult exclusively on acarids, the attack on these mites was slow and deliberate, and feeding was not commonly observed. Also, the larger acarids were apparently not subject to predation by the podocinids. The response to the presence of podurids, however, was rapid and aggressive. Stimulation of the sensory legs by a podurid resulted in an immediate attack, the insect being lifted off the substrate by the first one or two pairs of legs while the chelicerae were inserted for feeding. Often, in order to hold a particularly active podurid, the mite would rear backwards on its hind pairs of legs, lowering its opisthosoma to the substrate for balance. This effectively prevented the escape of the prey while feeding was initiated. This procedure was successful in a large percentage of the observed attacks, in spite of the great activity and agility of the springtails and their relatively large size. As soon as the more violent movements of the insect had subsided, the mite normally resumed walking, carrying the prey with the chelicerae. The anterior legs would continue their usual sweeping motion while the palps were held over the podurid, taking no apparent part in the feeding process. There was no evidence that any toxin might have been employed in the observed cases of feeding, since the collembolan would continue to struggle and move its legs as it was being drained. In some cases, especially when the prey was relatively unpigmented, it was possible to observe the protraction and retraction of the chelicerae in the insect, which occurred in alternate, left-right fashion. Feeding always occurred on the main part of the body, never on the appendages of the prey, although no particular portion of the body seemed to be favored. When the supply of podurids was regular, and the availability of prey in excess of the need, adults and large deutonymphs of *P. pacificum* tended to feed on half grown springtails, while protonymphs and young deutonymphs fed primarily on the smallest podurids available. The sizes of the Collembola as used here are expressed relative to the adult springtails, which may grow as large as 1085 μ, but which were usually less than 900 μ long. A half grown podurid is thus slightly longer than the body of the average adult podocinid, while the smallest Collembola, is no more than 250 μ long.

A mature female would normally complete feeding on a half grown podurid in 25-35 minutes, a one-third grown podurid in about 20-30 minutes, and a small podurid in about 10-15 minutes. Feeding by deutonymphs on one-third grown podurids usually took about 10-20 minutes, and on small podurids, 5-15 minutes. Protonymphs varied considerably in feeding time, 15 minutes being near the average, although the mite would often continue to carry the prey after the actual feeding was completed. In cases of the larger mites, half grown springtails were frequently not fully drained, although the younger mites tended to leave little more than the integument when finshed with a small collembolan.

When the podurid population was very large, it was found that larval or protonymphal podocinids may not survive, apparently due to crowding by the active Collembola.

The young podocinids also were found to be susceptible to death from starvation in cultures where the podurids were not reproducing and few small podurids or young acarids were present. This, in addition to observations of feeding behavior, suggests that the ability of the predators to locate prey at distances beyond the range of the sensory legs is extremely limited or absent. Results such as these imply that in nature, if the population density of acarids is sparse and adults predominate, the relatively long seven to eight day egg stage of *Tyrophagus* might prove to be critical to a predator with feeding requirements such as those of *P. pacificum*. It is possible that this may be a useful mechanism which offers some advantage to natural populations of acarids. In addition, for reasons such as these, it is likely that acarids and related mites are not the primary food for podocinids, the obvious preference for Collembola being further evidence to support this view.

When food is scarce, cannibalism is likely to occur among these mites. Adults have been observed feeding on all of the immature stages. Deutonymphal feeding on protonymphs is also not uncommon in crowded, starved cultures. On several occasions, an adult has been seen with its gnathosoma appressed to an egg; however, this position was not maintained after the cover of the chamber was removed for clearer observation. Once, an adult was seen carrying an egg with its mouthparts apparently in the same manner in which prey is carried. This egg was also quickly abandoned, however, it failed to hatch. Other evidence for egg cannibalism has been indicated when several eggs of different ages were left in a culture chamber without food. In such cases, if some of the protonymphs appear before all the eggs are hatched, some of the remaining eggs usually do not hatch and since non-viable eggs are normally uncommon, this suggests that egg cannibalism by the protonymphs may be involved.

The requirement for the presence of protonymphs to explain cannibalism is due to the fact that these mites are able to develop to the protonymphal stage without feeding, and atempts to obtain feeding by larvae on either small acarids or podurids have been unsuccessful. The ability of these mites to develop without feeding may be demonstrated by placing eggs in individual rearing chambers with no other organic material. The larvae from these eggs almost invariably develop into normal protonymphs with no difficulty. Strandtmann and Wharton (1958) note that a non-feeding larvae may occur in all species of the suborder.

Limited attempts to obtain feeding on alternate prey have largely produced negative results. Among the animals offered and not accepted have been two species of Bdellidae, one species of Cunaxidae, one of Scutacaridae, two of Stigmeidae, and five of Oribatei. Collembola were generally found to be readily eaten, and two species of Entomobryidae and three species of Sminthuridae have been taken by *P. pacificum.*

3. *Ecdysial Behavior*

As in other Mesotigmata, *Podocinum pacificum* does not exhibit any distinctive pre-molt behavior nor an akinetic chrysalislike stage as occurs in some of the trombidiform mites. Emergence of the larva from the egg, as well as ecdysis between any two instars, required several minutes and rarely took longer than ten. The process begins with the assumption of the ecdysial position, in which the walking legs are widely spread and the body slightly raised from the substrate. The sensory legs are extended directly forward. The ecdysial slit occurs anteriorly and laterally, separating the dorsum as an oval unit, which is hinged posteriorly. As the emerging mite withdraws its legs from the old skin, it works itself out in a postero-dorsal direction. The flaplike dorsum is thus pushed to a ventral position but remains attached to the rest of the skin which may be recovered as a unit when ecdysis is completed. In the larval to protonymphal ecdysis, the fourth pair of legs is normally the first to emerge from the larval skin, since they are not hampered by a closely fitted old skin as are the other legs. The anterior legs are generally the last to be retracted and tend to present the most difficulty to the mite in any of the molts due to their extreme length. The ecdyses observed were invariably terminated by a short period of grooming of the sensory legs, primarily concentrating on the densely setigerous apical portions and the long whiplike setae. The palps and chelicerae were used for this purpose, often aided by the second pair of legs.

Parts of the old skin remain attached when ecdysis is unsuccessful. Most frequently, this includes portions of the integument over the first pair of legs. Often the other legs are involved. In some of these cases, several of the legs might be bound together by the old skin, preventing normal locomotion, or small pieces of the integument may cover parts of the legs like stockings. It appears that such a condition results when the tarsi do not retain their normal position, firmly attached to the substrate, and become loosened, so that the mite is unable to withdraw the appendage from the old skin properly. The erratic behavior of such mites suggests that these tarsal coverings severely limit such sensory functions as might be localized there, inhibiting normal coordination. These mites, if immature, were unable to survive. When only small amounts of the integument remained, the mite could continue to live for as long as 19 days before death without evidence of an additional ecdysis. If enough of the skin persisted to render movement difficult, death usually occurred within one day, during which time no feeding occurred as the mite continued struggling to escape. This problem appears with greater frequency in the more crowded chambers and is probably a function of the increased likelihood of disturbance during the molting process.

In hatching, although not all of the eggs were viable, none of the larvae which began emergence was unsuccessful except in one series of experiments. This series was conducted to determine the percentage viability of the eggs and involved handling of a number of eggs with a water-brush. In some of these cases, the emerging larvae were either unsuccessful in completing emergence or required aid since they were in an inverted position and unable to contact the substrate. The infrequency of these inverted larvae suggests that only the nearly mature embryos are unable to compensate for the inversion of the eggs.

4. *Oviposition*

Several types of oviposition sites were used by the females of *Podocinum pacificum*, and a single adult might use all types. Both natural and artificial materials were used, and it appears that the physical shape or texture primarily determines the acceptability of a substance. Among the most commonly favored oviposition sites were threadlike fibers, such as the teased-out edges of fiberglass filter paper, or lint from tissue paper. Equally common was the use of finely pilose mats for egg laying. Two substances were available which provided such a surface; small shreds of fiberglass filter paper and a commonly-occurring type of mold which grows on yeast. The mold mycelia, however, often grew over and killed the egg. The parts of the threads to which eggs were attached are the raised portions which are not in contact with the substrate. The appearance of these elevated eggs is rather reminiscent of chrysopid eggs, and it is possible that a similar protective function is involved which reduces the likelihood of predation. In a crowded rearing chamber a single thread might bear as many as four eggs closely clustered together.

Among the less frequently used sites for oviposition are the bare substrate and pieces of dead leaves. Occasionally the egg would be laid on the substrate despite the availability of the fiberglass shreds. Although it was anticipated that bits of leaf litter would be preferred for egg laying, this was not found to be the case, the use of the substrate being more common. Rarely, instead of the egg being present on the upper surface of a fiberglass shred or a piece of dead leaf, it was found attached to the underside. In crowded cultures, oviposition on the underside of the stender dish cover became more frequent. Attempts to obtain large quantities of eggs from crowded chambers by offering 5 mm square pieces of paper toweling for egg-laying were initially unsuccessful. No eggs were laid on these in a period of four days, in two cultures of over 15 mature females each. After six days, however, three eggs were present on one piece of paper. It is possible that the presence of the first egg increases the attractiveness of a site for additional oviposition. This might explain the above mentioned clumping of eggs on the threadlike fibers. Experience with individual adults of this species indicates that this was probably not the result of a succession of eggs from a single female.

A series of studies was conducted using mature females to determine the average rate of egg production. The randomly selected individuals were divided into groups of six to 12 mites. These were well fed during the course of the observation period, a minimum number of 30-50 medium-sized Collembola being maintained in the chamber during the study. A total of 249 eggs was collected in a period of 563.7 female-days. The average rate of egg production was 0.455 eggs per female per day. The maximum short-term rate observed in individual cultures was 14 eggs in a ten day period for a single female. To approximate the actual rate of reproduction, it was noted that in a series of 151 eggs, 17 (11 %) were infertile. Mold, cannibalism and other factors which could be detrimental had been minimized although it is possible they were not totally excluded.

5. *Life History*

Egg. Ovoid; milky opalescence; chorion, thin, translucent; length, 217-225 μ; width, 167-184 μ; mean of 7, 219 × 175 μ. The eggs hatch after 12 to 48 hours, the mean for 30 eggs being 22.8 hours.

Larva. The six-legged larva is a reclusive, sedentary mite, with a distinctively rotund body. It normally stays near the egg after hatching, concealed in a depression in the substrate or under a piece of debris. When walking the larva is slow and deliberate even when disturbed. The sensory anterior legs, which appear to be of aid in balancing, are relatively shorter than in post-larval stages.

Body length, 180-286 μ; width, 194-234 μ; average size for 7 mites, 241 × 211 μ.[2] The gnathosoma, as measured from its postero-ventral edge to the tip of the hypostome, is an additional 63 μ. Pedipalps, 93-110 μ, measured from the ventral base of the trochanter. The palpal trochanter, femur and genu bear zero, 4 and 5 setae respectively. Chela stout, fixed digit with 3 teeth, pilus dentilus; movable digit with 1 tooth. Sparse brush of small hairlike excrescences around base of movable digit on the external (ventral) side where they may possess a proprioceptive function.

Tectum consists of 3 small, convex swellings bearing numerous small denticles (Fig. 2). In addition to the 3 pairs of long opisthosomatic setae described by DeLeon, the dorsum of the larva bears 8 anterior pairs of minute setae about 3 μ long (Fig. 1). The 3 long, posterior pairs of setae from anterior to posterior are 42-48, 41-48 and 30-34 μ respectively. No pores, tubercles or cicatrix areas on the dorsum. Ventrally, peritremes and lyriform pores not visible. Four pairs of setae anterior to 3 anal setae.

Tarsus I, 149-163 μ; terminal setae, 144-180, 192-204 μ; elongate subterminal setae 108-120, 113-120 μ. Leg I, 340-433, leg II, 227-330, leg III, 258-309 μ. Spiculate setae only on the 3 subapical segments (femur, genu and tibia) of the legs and limited to the dorsal surfaces of these segments. The fourth pair of legs of the protonymph may be seen through the integument of some of the mounted specimens of the larva.

This stage usually lasts 12-30 hours, the average for 35 mites being 20 hours.

Protonymph. The moderately active protonymph is the first feeding stage on this species. It is narrower and more depressed than the larva. In proportion to the body the legs are all more elongated than in the previous stage. The stiltlike habit of walking, with the body well raised from the substrate, as well as general carriage of the protonymph is similar to that of both the deutonymphs and adults. Previously this stage has been undescribed.

Body length, 234-302 μ; width, 182-256 μ; average of 8 mites, 273 × 210 μ. Gnathosomal average, 75 μ. Pedipalps, 129-130 μ; setae, 1, 4, 5. Chela, more slender than in larva (Fig. 7), fixed digit with 2 prominent teeth, movable digit with 4-5 teeth and pilus dentilus.

Tectum, tripartite; lateral prongs with several small external teeth; medial prong with 2-4 terminal teeth (Fig. 5). Dorsum with 16 pairs of setae; paravertical setae and 2 posterior pairs large and spiculate. Three pairs of medium sized dorsal setae immediately behind level of fourth coxa are also sparsely spiculate (Fig. 3). Paravertical setae, 58-65 μ, 2 pairs of large posterior spiculate setae, 89-101, 67-77 μ. Dorsally, a pair of porelike structures behind paravertical setae and a second pair at level of coxa IV close to the lateral margins of dorsum.

Venter: 3 pairs of sternal setae, 3 pairs anterior to the 3 anal setae, 1 pair lateral to the anal setae (Fig. 4); 2 pairs ventral pores, 1 pair mesad of coxa IV, 1 pair laterad of the anus. Peritreme short, extending from level of coxa IV to posterior edge of coxa III.

While the body integument of the protonymph is generally smooth, an area of minute toothlike structures, similar to those on the larval tectum, is present just posterior to the anus.

Tarsus I, 187-214 μ, 2 terminal setae 250-270 μ, 187-196 μ (Fig. 6). Subterminal setae not distinctively elongated. Leg I, 593-666 μ; II, 374-395 μ; III, 343-364 μ; IV, 406-447 μ. The distribution of spiculate setae on the legs of the protonymph is similar to the pattern described for the larva.

For 32 mites this stage lasted 3.5 days, varying from one to ten days. Those individuals which underwent ecdysis after less than 20 hours or more than 120 hours did not survive beyond the next stage.

Deutonymph. The deutonymph of *P. pacificum*, although very similar to the previous stage, may be distinguished by an added seta on the palpal trochanter, femur and genu, resulting in a 2,5,6 formula.[3] The gnathosoma averaged 98 μ for 8 mites; palps 137-156 μ. The chela and tectum of the deutonymph do not differ substantially from those of the protonymph.

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[2] These measurements were recorded from preserved, mounted specimens. The range of sizes has thus been unavoidably exaggerated due to flattening and distortion in the mounting process.

[3] Five setae on the palpal genu was reported by Evans and Hyatt.

Body, 260 × 182 — 527 × 260 μ, average of 8 mites, 333 × 226 μ. Excluding a pair of postero-ventral setae which is probably homologous to DeLeon's "interscutal setae" borne close to the dorsal shield in the adult, the dorsum may be considered to bear 16 pairs of setae[4]; the paraverticals and 2 pairs of posterior dorsal setae elongated (Fig. 8). All except 2 pairs of the dorsal setae are spiculate, the exceptions being the vertical and the first anterior lateral pair of setae. Long opisthosomal setae, from anterior to posterior, 125-156, 101-120 μ.

Dorsally, occasional specimens exhibit the cicatrix areas which are conspicuous among adults. A pair of pores located between the 2 sets of elongated opisthosomal setae of the deutonymph probably corresponds to pores in the same area in the adults.

Venter with 4 pairs of sternal setae, 1 pair in the genital region (at the level of coxa IV), 3 pairs anterior to 3 anal setae, 1 postanal pair at the weakly defined postero-lateral edge of the ventri-anal shield. Three pairs of lyriform pores associated with the sternal setae are sometimes visible. Densely denticulate area surrounding the medial anal seta, and a similar, more sparsely denticulate area between the posteriormost pair of median dorsal setae. Peritremes extend anteriorly from the level of coxa IV ventrad of the lateral margin of the dorsum but turn dorsally at the level of coxa II and follow the anterior edge of the dorsum, usually terminating immediately mesad of the lateral gnathosomal margins.

Tarsus I, 240-293 μ, terminal setae 278-340, 216-278 μ (Fig. 9). Leg I, 772-978 μ; II, 433-515 μ; III, 391-515 μ; IV, 474-618 μ. The sparsely distributed spiculate setae of the legs primarily on dorsal surface of middle segments of 3 posterior pairs of legs.

Ecdysis occurs after 2-7 days, the average for 33 mites being 3.6 days. One mite, which died without undergoing a final ecdysis, survived 18 days as a deutonymph.

Adult. Females generally oviposit within 1 week after the terminal molt. The average pre-oviposition period for 26 adults was 7.3 days, although some females began laying in the third day, while one did not begin until after 17 days. The longest egg-laying period for a *pacificum* female was 29 days, while the greatest number of eggs produced by a single adult was 10. Two different females are responsible for these records. Under normal conditions, the average number of eggs per female for 13 adults was 6.

Podocinum sagax

Podocinum sagax (Berlese). 1882a. Bull. Soc. ent. ital., *14*: 340.
Laelaps sagax Berlese, A. 1882. Atti Ist. Veneto (5), 8, 1: 638.

Distribution of this species includes the following: ITALY (Berlese, 1882, 1913); GREAT BRITAIN (Turk, 1953) (1); INDONESIA. *Bogor:* 10-12 Jan. 1954, A. H. G. Alston (1); ARGENTINA. *Tucumán:* 800 meters, Parque Aconquija, Jan.-March 1953, P. Wygodzynsky, rotting leaves (1); PANAMA. *Canal Zone,* Barro Colorado Island, 22 Feb. 1955, C. W. Rettenmeyer, grass and roots (3); WEST INDIES FEDERATION. *Jamaica,* St. Ann, 1,500′, Mt. Diablo, 3 June 1956, P. F. Bellinger, leaf litter on limestone (1); PUERTO RICO. *Quebradillas,* 4 July 1951, J. M. Capilles, on quajatzca (2); UNITED STATES. *California:* San Francisco, imported from China, 22 Dec. 1937, on *Zingiber officinale* (2), imported from Guatemala, 31 July 1936, R. Clemens, on debris with *Odontoglossum grande* (2); *Texas:* Gonzales Co., Palmetto State Park, 5 April 1954, W. T. Atyeo & J. G. Borland, palmetto beating (3), 24 April 1960, G. Singer, under board in wet woods (3); Brownsville, imported from Guatemala, 9, 17 Sept. 1946, on orchid plants (2); *Louisiana:* Jefferson Co., Harahan, 31 Oct. 1944, F. G. Werner, under log, on bark (5); *Florida:* Highlands Co., Highlands Hammock State Park, 8 April 1962, C. L. Wong, rotten hardwood log and leaf litter in wet oak-palm woods on shallow loamy soil over fine sand (3). This collection provided all the original living specimens of *P. sagax* used in this study.

BIOLOGICAL DESCRIPTION

1. *General Notes*

Podocinum sagax closely resembles *P. pacificum*; however, the females are darker, appearing to be more strongly sclerotized and more robust-bodied than those of *P. pacificum*. This may be partly due to a greater number of eggs carried by a gravid *sagax* female which results in a relatively more

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[4] Fifteen pairs of dorsal setae were illustrated by DeLeon, his figures differing in the absence of the pair of setae directly behind the paravertical setae.

enlarged opisthosoma and a roughly triangular overall appearance. The actual size of 5 females of *P. sagax* in life was 417-501 μ long exclusive of the gnathosoma, and 282-375 μ wide. The first pair of legs are 1503-1670 μ long. In addition to the apical sensory setae, the tarsi of the long anterior legs each bear a pair of of subterminal setae, one-third to one-half as long as the terminal setae. These are perpendicular to the long axis of the tarsus and held parallel to the ground so that under moderate light and magnification they are often visible. Since these prominent setae are present in all stages of *P. sagax* and absent in all except the larval stage of *P. pacificum*, most of the members of the two species can be differentiated on the basis of this character in life. Also, under strong light and high magnification, the randomly distributed punctations on the dorsum of the adults of *sagax* can be distinguished from the reticulated pattern present in *P. pacificum.*

The males of *P. sagax* may be identified by their distinctive, narrowly oval shape, which is similar to that of young, non-gravid females. Young females however are neither as strongly armored nor as prominently grooved dorsally as the males. In addition, males are usually slightly smaller than the young females.

In activity, *P. sagax* is a reticent mite; however, when disturbed, it is quicker and more agile than *pacificum.* Stimulation by exposure to ordinary room lighting usually results in moderately active walking near the periphery of the stender dish culture chamber. In the plastic box type of rearing pen, activity is reduced after a short time, and the mites may be found resting usually near the corners of the chamber.

As is the case with *pacificum*, under uncrowded conditions, most of the stages of *sagax* do not use the paper shreds or bits of leaf litter as hiding places either when resting or when disturbed. The larva, however, which is the least active of the post-embryonic stages, is likely to take advantage of the protection offered by foreign bits of material or depressions in the substrate. With an increase in the density of the *Podocinum* population, the frequency with which the post-larval stages may be found under pieces of leaf or other extraneous matter also increases.

The responses exhibited by *P. sagax* to stimulation by a moist camel's-hair brush, contact with free water, or contact with another *Podocinum* are similar to those described for *P. pacificum* under the same conditions.

2. *Feeding Behavior*

The primary difference in feeding behavior between *P. sagax* and *pacificum* is the more aggressive activity and apparent voraciousness of *sagax* females. While the feeding technique is not significantly different between the two species, all the post-larval stages of *sagax* are likely to attack larger podurids than the corresponding stage of *pacificum.* As a result, *sagax* was frequently observed to bend over backwards, apparently supported only by the fourth pair of legs and some of the opisthosomal setae, in order to lift a relatively large and active collembolan from the substrate. In this species also, there was no evidence of the use of a toxin in overcoming the prey.

In feeding, a mature female would usually drain a half-grown podurid in 10-20 minutes, while a half-day old male might take as long as an hour to drain such a podurid. A protonymph, feeding on a proportionately smaller springtail, would take 5-15 minutes from start to finish. In one instance, a mature protonymph was observed to feed on a three-quarters grown podurid, taking about two hours and 45 minutes, during which time only the head of the prey was drained. In this case, the legs of the insect continued to twitch at least one and a half hours after the initiation of feeding.

The method of attacking acarids, while similar in the two podocinid species, is different from that used against the Collembola. Once an appropriate acarid is encountered, the predator follows behind, attempting to insert the chelicerae into the retreating acarid. During this time, contact is maintained either by the palps alone, or with the aid of the sensory legs. Occasionally, these legs will be folded backwards and remain unused throughout the feeding process. If the predator is able to insert its chelicerae, these are then used to lift the acarid off the substrate for feeding.

In most of the observed cases of predation by *P. sagax*, the mite alternated between resting in one place for several minutes with walking at a moderately rapid rate, while carrying the prey supported by the chelicerae. Observation of a portion of the dorsal shield which pulsates during feeding suggests that the bald or "cicatrix-like areas" (DeLeon) represent the location of muscle origins, the large middorsal pair of areas marking the cheliceral retractors. Histological sections of *P. sagax* have confirmed the attachment of these muscles along the midline at the level of the fourth pair of legs. Other bald areas, primarily antero-laterad of those of the cheliceral retractors, probably locate leg and palpal muscle origins.

In the final stages of feeding, only the deflated integument of the prey is normally left, and although some pre-digestion may have occurred, the strenuous cheliceral activity is probably sufficient to account for the total mastication of the internal structures of the prey. Occasionally, *sagax* appeared to continue to use its chelicerae in alternate scissorslike fashion to shred and perhaps consume some of the remaining integument. Feeding normally concluded with a short period of rotation of the carcass, by palpal manipulation. The remaining ball of skin would be dropped, and cleaning of the chelicerae would begin.

Cannibalism also has been recorded in this species, egg protonymph and deutonymph being susceptible. It is probable that the reclusiveness and relative inactivity, as well as the shortness of the larval period, reduce the predation on this stage which appears quite defenseless otherwise. The eggs, however, seem to be less protected, as suggested by the increase in non-viable eggs in crowded cultures. In one case, a protonymph was observed feeding on a *sagax* egg, carrying it with the mouthparts in the conventional manner. The cheliceral activity was visible through the semi-transparent chorion, and partial deflation of the egg took place during a period of approximately 10 minutes.

In this species also, larval feeding has been unrecorded although small acarids and Collembola and their eggs have been offered. These larvae have likewise exhibited the ability to develop to the protonymphal stage in individual cultures in the absence of organic material. The post-larval stages have been observed to feed on eggs of *Neobeckerella.*

Alternate living prey, which were left in semi-starved cultures for a minimum of two days without being eaten, include one species of Bdellidae, one of Tetranychidae, one of Scutacaridae, two of Stigmeidae, and five of Oribatei. One adult female was observed attempting to feed on a tetranychid; however, insertion of the chelicerae was apparently unsuccessful, and after several exploratory probings, the attack was discontinued. Insects of one species of Entomobryidae and two of Sminthuridae, as well as one acarine species of the family Ascidae have been attacked and eaten by *P. sagax.*

3. *Ecdysial Behavior*

Ecdysis in *P. sagax* is similar to that of *P. pacificum* in all essential aspects including duration. Close examination of the process in this mite suggests that the ambulacral claws, which are present on all of the legs except the first pair, may be used to anchor the appendages to the substrate during ecdysis. Experience in removing newly shed skins from the substrate further supplements the observation that the apices of the tarsi are the primary points at which the old integument is fastened to the substrate. In the absence of ambulacra on the first pairs of legs, curved or hooked terminal setae may fulfill the function of attachment. In *P. sagax* and *P. pacificum*, all post-larval stages bear a strongly bent or recurved seta on the apex of tarsus I (Figs. 6, 9). Larvae of these species have slightly curved setae in this position; however, since the anterior legs are relatively short, these setae may not be used in ecdysis. All post-larval stages illustrated by DeLeon and by Evans and Hyatt exhibit similar curved or hooked apical setae, the pretarsi being uniformly absent in the family.

As with *P. pacificum*, upon completion of ecdysis the palps and chelicerae apparently clean and groom the tarsi and terminal setae of the anterior legs. In *sagax*, this has been followed in some cases by a period of several minutes during which a small portion of the old integument is maniputed by the chelicerae. This does not appear to be a feeding activity, and in fact, neither discarded egg shells nor shed skins seem to be eaten by any of the stages of either species.

4. *Sex and Oviposition*

Prior to this study, out of 28 specimens of podocinids in the Kansas University Snow Entomological Collection, only a single male of *P. sagax* was present. The only males recorded by Evans and Hyatt (1958) in this family are three individuals of *Podocinum aciculatum* from Nepal. They note, however, that Berlese (1882b) figures a male of *P. sagax*.

The infrequency of occurrence of males among the 14 species of podocinids raises the question of their role when they are present.

Of the nine live mites in the original collection of this species which were used in this study, three were males and six were females. Initially, these males were unrecognized as such, since I had not previously observed living males and since work with *P. pacificum* demonstrated that parthenogenic reproduction might be expected in this family. It soon become evident that these three individuals were unusual when they did not become gravid after nine days while the other adults became filled with eggs whether or not they oviposited. Mounting one of the three mites for microscopic examination confirmed that these were males. Once having identified a male, it became relatively easy to distinguish between the sexes by the difference in shape and degree of apparent sclerotization.

One of the remaining males was then introduced into a chamber with a female, which although gravid, had failed to oviposit during the nine days in culture. The male wandered around near the periphery of the dish for about five minutes before encountering the female, apparently by chance. Upon contacting the female with the sensory setae of the front legs, a short period of mutual stroking with the anterior legs followed for about half a minute. At the end of this time, the male proceeded to mount from behind onto the dorsum of the now quiescent female. Continued stroking of the front legs occurred for about half a minute whereupon the male turned 180° and crawled under the female, assuming a venter-to-venter position, both mites facing in the same direction. After another half minute in this position, the male moved away, and sperm transfer was assumed to have been completed. Three minutes later the female was observed to feed normally on a podurid and about 15 minutes after the first mount, mating was again attempted. This time, contact was discontinued after the male had rested on the dorsum of the female for half a minute. In this instance, the female died two days later without evident cause and without ovipositing. It is possible that a prolonged gravid period before mating had a detrimental effect although other females showed a tolerance to this condition.

It was soon found that the mating behavior usually occurred fairly readily when a male was placed near a virgin female or a female which had not been recently mated. Semistarved or newly-emerged adults, however, were not observed to mate, apparently being less responsive to sexual contacts than is usual. In each of the observed cases, the total duration of the mating process was about one and one half minutes. The assumption that sperm transfer actually occurs within this period was supported by studies in which the females were isolated after this single contact. In such cases, the females are capable of laying eggs after the usual pre-oviposition period, the resulting offspring including members of both sexes.

In normal mating, the typical response of the female to stroking of the anterior legs is an inhibition of movement thus permitting mounting by the male. It was therefore thought that the sensory legs might be critical to proper mating behavior in the female. However, with a female whose anterior legs had been amputated several observed attempts at mating were unsuccessful, but when the male was left in the rearing chamber with the female over a period of days, normal offspring were produced. These results have proved to be repeatable.

While a *sagax* female, raised in a rearing chamber with one or more males, normally began oviposition three or four days after the final molt, this pre-oviposition period could be prolonged by preventing contact with a male. As a result of maintaining unmated females as long as 26 days without egg production, it is concluded that unmated reproduction probably does not occur in this species. That the mites used to test this hypothesis were, in fact, capable of producing fertile eggs was also tested. In one case, two males were introduced after a 23 day eggless period, and in a second case, two males were added after a 26 day pre-oviposition period. In each case, although the virgin female had become gravid within one week of the final molt, a post-mating, pre-oviposition period of three days preceded the production of eggs. The persistance of the pre-oviposition period led to the hypothesis that mating in each of these cases represented an actual contribution of genetic material by the male, rather than being simply a physical stimulus necessary to elicit egg-laying. This view was further supported by the fact that the offspring from these matings include members of both sexes.

In general, those females whose pre-oviposition period had been experimentally prolonged were found to mate less readily than females which were mated within one week of the terminal molt.

In addition to the evidence that oviposition does not occur unless the female has been mated, it appears that mating must be repeated in order for the females to continue egg production. While a mated female may continue to lay eggs for as long as ten days after the removal of males from the chamber, egg production is eventually terminated and has not been known to resume until a minimum of three days after the reintroduction of a male. During the entire eggless period and the post-mating pre-oviposition period, the female will be conspicuously gravid. The highest short-term rates of oviposition occur among females with artificially prolonged pre-oviposition periods, the maximum being 13 eggs produced in a four day period. In this case, after a three day post-mating pre-oviposition period, the first six eggs were produced in less than 24 hours. The oviposition rate for *P. sagax* under normal conditions was determined using groups of seven to ten females. Three or more males were included with each group of randomly selected mature females. The well-fed females produced a total of 350 eggs in a period of 504.8 female-days. The average number of eggs per female per day was 0.69. A further study of egg survivorship showed that out of a total of 246 eggs examined ten eggs, or four percent, were not viable.

As with *P. pacificum*, the frequency of use of a particular type of oviposition site by *P. sagax* varied with the degree of crowding in a chamber. In the stender dishes with single females, or groups of less than six, the mites would generally be present on the substrate, and eggs would rarely be found on the walls or cover of the chamber. Patches of finely pilose mold or shredded fiberglass filter paper and cellulose threads from tissue paper were commonly selected as oviposition sites under these conditions. Other acceptable thread-like materials include human hair and strands of woolen yarn. Less frequently, the eggs are laid directly on the substrate. Under crowded conditions, the occurrence of eggs on the walls and on the underside of the stender dish cover becomes increasingly common. This appears to be associated with the more frequent use of these areas as resting places by both males and females. The majority of eggs in such cases will be on the underside of the cover. The adhesive character of the chorion is also evident in this species. When the small plastic boxes are used as rearing chambers, eggs are most frequently laid on the walls of the container, both under crowded and uncrowded conditions.

The tertiary sex ratio was studied by selecting all eggs laid within a given period and determining the sex of all emergent adults. Several replicates, totaling 158 eggs, gave the ratio of 75 males to 83 females, suggesting a primary sex ratio near 1:1.

Preliminary egg squashes of this species show chromosome numbers of five and ten. Since the eggs of *P. pacificum* appear to have ten chromosomes, it is likely that a haplo-diploid sex determining mechanism occurs in *P. sagax* with the males being haploid and the females of both species having the diploid chromosome number of ten. Females of *Dermanyssus gallinae* (Dermanyssidae) similarly must be mated in order to reproduce, the males being haploid according to Oliver (1962), who notes that a haplo-diploid type of sex determination is common among the Mesostigmata.

5. *Life History*

With the exception of the egg and larval stages, *P. sagax* can be readily distinguished from *P. pacificum* on the basis of the chaetotaxy of the dorsum and anterior legs. The stages of *sagax* may be differentiated on the basis of the palpal chaetotaxy, the formula being the same as that of the corresponding stage of *pacificum*.

Survivorship among the various stages was also studied in this species, a total of 74 mites being used in the survey. Under uncrowded conditions, with access to adequate food, five eggs did not hatch, two individuals died as larvae, 16 mites died during the protonymphal stage, six during the deutonymphal stage, three during the terminal molt, and 42 survived to adulthood. Due to segregation of different sized individuals, none of these deaths were likely to have been caused by cannibalism except possibly among the eggs. The high mortality among the protonymphs is interpreted as being a function of their size and strength which makes them unable to overcome and feed on any but the smallest collembola and acarids. Their thin, untanned integument and high surface-area to volume ratio may hasten dehydration and increase the frequency of death by desiccation.

Egg. In appearance, the eggs of the 2 species of *Podocinum* are identical. Ten eggs of *sagax* were 200 × 159 to 233 × 184 μ, mean, 216 × 172 μ, which is extremely close to *pacificum*. The length of this stage for 45 eggs was 6-58 hrs., the average 32.5 hrs.

Larva. The larvae of the 2 species are also identical, the long subterminal setae of the anterior legs, which are typical of all stages of *sagax*, being present only in this stage in *pacificum*. Although the majority of individuals are sedentary, the larvae of *sagax* are generally more active than those of *pacificum*.

This stage is 208 × 156 to 286 × 218 μ, the mean for 8 larvae, 250 × 185 μ.[5] The gnathosoma averages an additional 55 μ. The pedipalps 108-137 μ. The chaetotactic formula for the palpal trochanter, femur and genu is 0, 4, and 5.

Chela; fixed digit with 3 inconspicuous teeth, reduced pilus dentilus; moveable digit bears a single low tooth.

As in *P. pacificum*, the tectum little developed and of 3 small lobes bearing many minute denticles. Dorsum of the larva bears 11 pairs of setae, of which the anterior 8 pairs are reduced, ca. 2 to 3 μ long, set in setal bases approximately 3 μ in diam. (Fig. 10). From anterior dorsal to posterior ventral, the 3 pairs of elongate, simple opisthosomatic setae are 48-58, 38-52, 36-46 μ.

Pores, tubercles and cicatrix areas not visible on mounted specimens. Four pairs of setae on the venter of larva anterior to the 3 anal setae (Fig. 11). Peritreme not visible at this stage.

Tarsus I, 140-160 μ bearing terminal setae 192-226, 156-180 μ, subterminal setae, 127-161, 154-180 μ long. Leg I, 381-412 μ; leg II, 257-278 μ; leg III, 227-257 μ.

While all the body setae are simple, spiculate setae occur on the legs, and are located on the dorsal surface of the femur, genu and tibia. A proximal to distal increase in number of setae per segment occurs on all legs.

Ecdysis occurs after 10-14 hours with 12 hours as the mean for 45 larvae.

Protonymph. This is a quick-moving, actively feeding stage. It is, however, quite sensitive to unfavorable environmental conditions and survivorship is lowest in this stage. Eight protonymphs varied from 234 × 182 - 390 × 286 μ and averaged 299 × 255 μ; gnathosoma, 75 μ. Palps, 127-132; basal segments bear 1, 4 and 5 setae on the trochanter, femur and genu respectively.

The 3 pronged tectum is similar to that of *p. pacificum*, external teeth on the lateral prongs, the medial prong distally 2-3 branched.

Chela; fixed digit, 4-5 teeth, pilus dentilus; movable digit, 2 teeth.

The dorsum of the protonymph bears 17 pairs of setae differing from *P. pacificum* in having 1 additional pair of medial setae (Fig. 12). Although variable, 6 pair of posterior dorsal setae commonly spiculate as well as the paravertical setae. Anterior pair of long opisthosomal setae, 53 μ; posterior pair, 56 μ. Cicatrix areas not visible but a minutely denticulate region of integument present mesad and posteriad of long opisthosomal setae. A similar roughened area present ventrally, posterior to the anus.

The venter bears 3 pairs of setae in the sternal region, 3 pairs anterior to the anus and 1 pair lateral to the 3 anal setae. Two pairs of porelike structures present, 1 near the posterior margin

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[5] These measurements were also obtained from slide-mounted specimens.

of coxa IV and 1 pair lateral to the anus. Peritreme short, 29 μ, from coxa IV to the posterior margin of coxa III.

Tarsus I, 211-228 μ, bears terminal setae 225-240, 293-312 μ and subterminal setae, 115-125, 125-132 μ. Leg I, 614-655 μ; leg II, 374-406 μ; leg III, 364-374 μ; leg IV, 395-426 μ. Spiculate setae of legs sparsely distributed on dorsal surface of middle segments of 2 hind pairs of legs while few or none present on ventral surfaces of segments or on legs I and II.

Individuals which successfully undergo ecdysis usually do so within 42-82 hours of the protonymphal emergence, the average for 45 mites being 50.5 hours.

Deutonymph. The deutonymph is also an active and frequently-feeding mite, apparently being more voracious than *P. pacificum* in the corresponding stage. Body, 312 $\times$ 208 - 390 $\times$ 286 μ, mean 352 $\times$ 242 μ, gnathosoma, 108 μ in 8 deutonymphs. Palps, 154-161 μ; trochanter, femur and genu bear, 2, 5, and 6 setae, as in the adults. Distribution of cheliceral teeth the same as in protonymphs. Tectum 3 pronged as in the protonymph, the middle prong terminating in 2 to 4 points.

18 pairs of dorsal setae result from addition of a pair of postero-lateral setae which probably corresponds to the "interscutal setae" borne near or on the dorsal shield of *p. pacificum* (Fig. 13). Anterior pair of long opisthosomal setae, 65-74 μ; posterior pair 74-84 μ. A pair of pores present between these pairs of elongated setae. Altogether 7 pairs of dorsal setae are spiculate. Cicatrix areas usually not visible but numerous tubercles distributed over posterior half of dorsum. Anteriorly and laterally, margin of dorsal shield may be recognized by the absence of the fingerprintlike striations of the conjunctiva.

Ventrally, the deutonymph bears 4 pairs of setae in the sternal region, 1 pair in the genital region, 3 pairs of preanal setae posterior to coxa IV, a pair lateral to the anus, and 3 anal setae. A small, crescent-shaped band of denticles immediately posterior to anal setae. Sternal lyriform pores only rarely seen in this stage. However, a pair of minute pores typically within triangles formed by 3 posteriormost ventral setae laterad of the anal setae. Peritreme extends anteriorly from spiracular plate in the region of coxa IV, turns dorsally in the region of coxa II, and follows margin of dorsal shield terminating slightly laterad of the paravertical setae.

Tarsus I, 283-291 μ; terminal setae 260-291 μ, 291-351 μ. Subterminal setae, 139-149, 158-170 μ. Leg I, 884-926 μ; leg II, 506-530 μ; leg III, 417-489 μ; leg IV, 541-563 μ. Spicules reduced in size and frequency among the setae of the legs.

Ecdysis occurs after 46-82 hours, with an average of 52.7 hours for 40 mites. The total egg to adult period for this species is 122 to 194 hours, or 5.1 to 8.1 days. For 27 females, this period averaged 6.6 days, while for 18 males the average was 6.2 days. The difference of 0.4 days which is primarily due to the lengths of the egg stage was not found to be statistically significant.[9]

Male. 364 $\times$ 312 - 416 $\times$ 364 μ, the average of 6, 390 $\times$ 329 μ with the rostrum, 103 μ. Anterior legs, 1222-1300 μ long, with an average of 1261 μ. The legs are thus proportionately slightly longer than those of the female.

The average life span of 12 males which appeared to die of "natural" (i.e. physiological) causes was 23.5 days, with a range of 9 to 41 days. The standard deviation for this group was 10.2 days.

Female. Normally, after a 3 to 5 day pre-oviposition period, a newly hatched female in culture with a male would begin a period of egg-laying which could last as long as 36 days. The greatest number of eggs produced by a single female has been 38 over a 23 day period. In a few cases, where females appear to have produced all the eggs of which they are capable, the post-oviposition period was 2-16 days, with the average 6.6 days for 5 females. The maximum adult female life span recorded is 88 days. The female concerned had its pre-oviposition period experimentally prolonged, and evidence from other females suggests that such treatment may frequently result in an appreciably longer adult life span. The average of 20 females which seemed to die "naturally" was 50.9 days for the total adult life span. Here, the standard deviation was 22.7 days. Thus, the life span of the female is considerably longer than that of the male, and indeed, the males even appear more susceptible to death by desiccation as well as starvation than the females. Male survivorship may therefore prove to be one of the more important factors which determines the limits of distribution of this species, the ability of individual males to fertilize several females being dependent on population densities and, in all likelihood, random encounters.

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[9] At 5% confidence limit using Wilcoxan's non-parametric ranking method.

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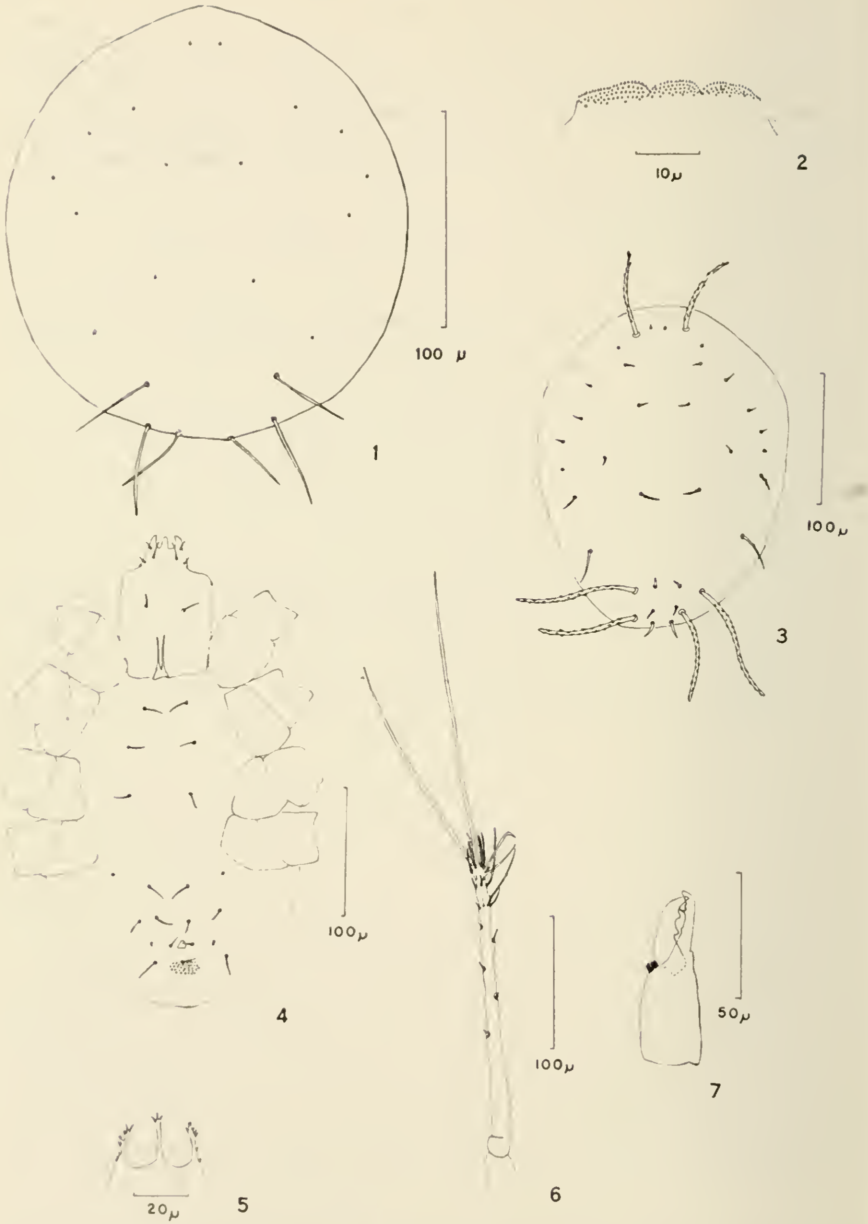
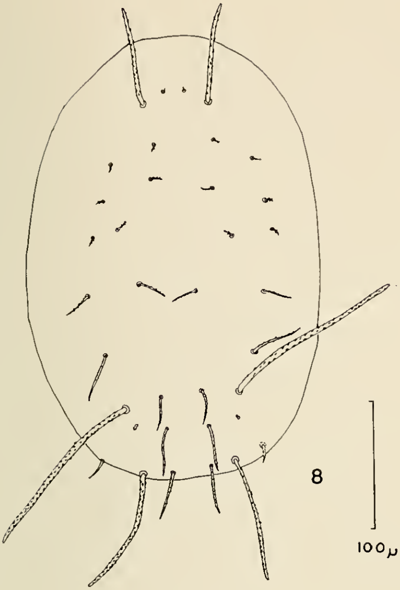


FIGS. 1-7. *Podocinum pacificum* Berlese. FIG. 1. Larva, dorsum. FIG. 2. Larva, tectum. FIG. 3. Protonymph, dorsum. FIG. 4. Protonymph, venter. FIG. 5. Protonymph, tectum. FIG. 6. Protonymph, left tarsus I, ventral aspect. FIG. 7. Protonymph, left chela, lateral aspect.



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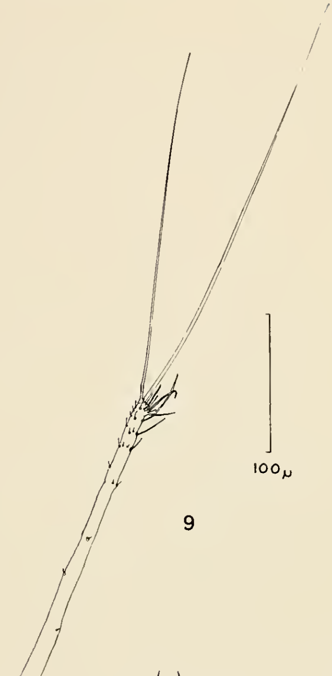

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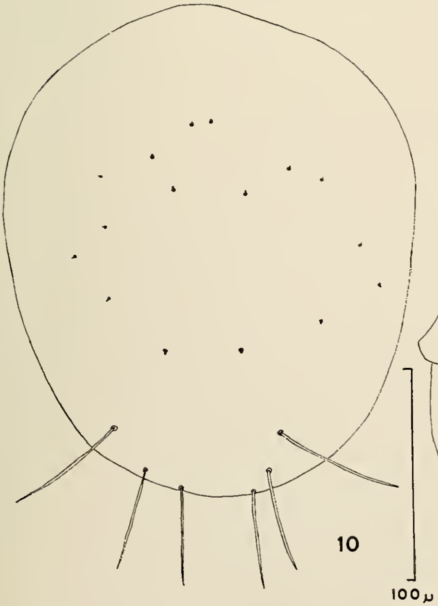

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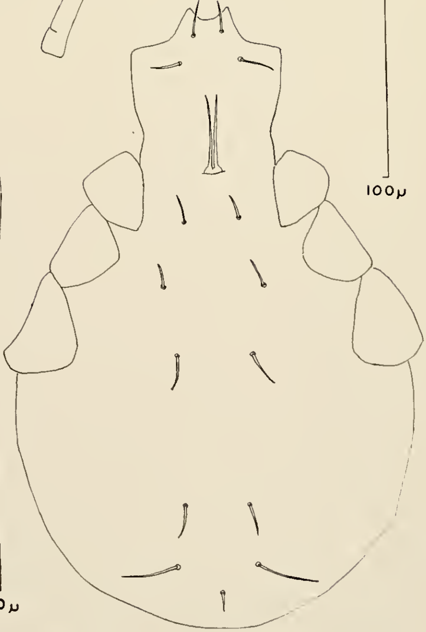

FIGS. 8-11. *Podocinum pacificum*. FIG. 8. Deutonymph, dorsum. FIG. 9. Deutonymph, left tarsus I, ventral aspect. *Podocinum sagax* Berlese. FIG. 10. Larva, dorsum. FIG. 11. Larva, venter.

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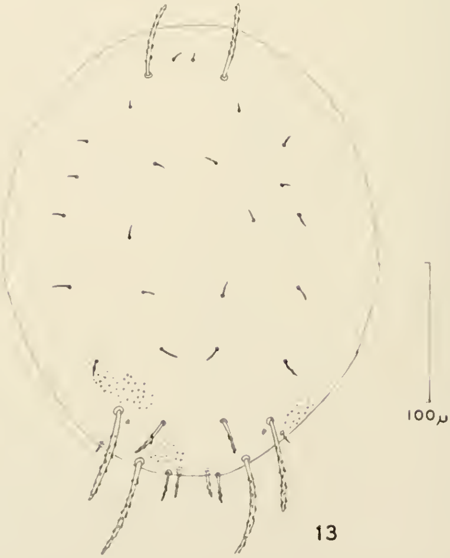

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FIGS. 12, 13. *Podocinum sagax*. FIG. 12. Protonymph, dorsum. FIG. 13. Deutonymph, dorsum.

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SCIENCE BULLETIN

THE ERIOPHYOIDEA OF KANSAS

By

C. C. Hall, Jr.

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The *University of Kansas Science Bulletin* (continuation of the *Kansas University Quarterly*) is issued in part at irregular intervals. Each volume contains 300 to 700 pages of reading matter, with necessary illustrations. Exchanges with other institutions and learned societies everywhere are solicited. All *exchanges* should be addressed to

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The Eriophyoidea of Kansas*†

C. C. HALL, JR.[1]

ABSTRACT

This paper is a systematic account of the Eriophyoidea in Kansas. Information is also given on life histories, distribution, behavior, economic importance, and techniques of preparing specimens. Eleven previously described genera are included in this study. Of the 29 species included in this paper, 7 are new. The described species are *A. nimia*, *Phyllocoptes microspinatus*, *Phytoptus rotundus*, *Rhyncaphytoptus boczeki*, *Vasates cercidis*, *V. dimidiatus*, and *V. michneri*.

INTRODUCTION

This work is the result of four years of collecting of Eriophyoidea in Kansas, during which time the eastern part of the state was extensively sampled at all seasons and the western part during the summer months. Twelve genera and 29 species were found in Kansas. Keys to aid in the identification of species and descriptions and figures of all species included in this study are given.

Slykhuis (1953) is largely responsible for bringing about more interest in eriophyid mites in recent years. It was his discovery of *Aceria tulipae* (Keifer) as the vector of wheat streak mosaic disease which aroused new interest in the Eriophyoidea. This discovery, and the subsequent studies of diseases of Kansas wheat and their vectors, indicated an urgent need for more information on this economically important family of mites, and this study was launched to meet it.

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[1] Associate Professor of Biology, The University of Texas at Arlington, Arlington, Texas 76010.

Keifer's (1952) work provided the taxonomic framework and keys necessary for a survey of the Kansas Eriophyoidea. Prior to this there were no keys available and many previously described species were based upon inadequate descriptions. Host lists with good but incomplete figures have helped to make many of Nalepa's species recognizable. Nalepa (1911) gave the first reasonably adequate descriptions of mites in this group.

It is hoped that this investigation of Kansas eriophyids will form a nucleus of material that will be useful to taxonomists and those interested in agricultural or economic problems that involve the Eriophyoidea. Eriophyids have not been collected or studied previously in the Kansas area, and it is clear that many species besides those so far obtained must exist in the state. However, the material collected shows that Kansas is inhabited by several genera and many more species than was expected at the beginning of this investigation.

The needs of beginners in the study of eriophyids have been kept in mind during the preparation of this paper. The lack of detailed information on techniques of collecting and slide preparation has had a limiting effect on the number of species that could be included in this study. Only recently have techniques and mounting media been such that the agricultural or survey worker can send prepared slides to the taxonomist instead of infested plant material.

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HISTORY

The formation of galls of various types on the leaves of plants was noted and investigated as early as 1737 by Reamur (Hassan, 1928). The small wormlike inhabitants of many of these galls were thought by Reamur to be insect larvae; it was Dujardin (1851), more than a hundred years later, who reported that they were actually adult mites. Landois (1864) gave further support to the belief that these wormlike organisms were mites. Cromroy (1958) gives a fairly detailed account of early students of eriophyids.

Much credit must be given to Alfred Nalepa whose work (1887-1929) was large enough and good enough to serve as the basis for eriophyid taxonomy as it exists today. Many species that he described must be determined by host relationships, since he figured only dorsal, ventral, and lateral views of whole mites. Such data are frequently sufficient to speculate with some accuracy on species identity, but without figures of legs, genitalic structures, featherclaw, and skin structure, one can never be absolutely certain of identifications.

Since Nalepa proposed the family Eriophyidae in 1898, about 1,000 species in approximately 78 genera have been described. Keifer and Nalepa have been the major contributors.

METHODS AND MATERIALS

Collecting: Collection of eriophyid mites is relatively easy but in Kansas is seasonal, with the best results in late summer. When foliage first appears in early spring, eriophyid populations are low; about six weeks, or one generation later, they are much more abundant and relatively easy to locate with a hand lens or dissecting microscope. A few species, especially those that form clusters of twigs ("witches'-brooms") or other abnormal growths, can be collected at any time of the year by examining these abnormalities. Species that produce galls of various types or marginally rolled leaves are not difficult to find, and eriophyids are usually abundant in these deformities. Types of galls and other plant distortions made by eriophyids have been studied by Schlechtendal (1916). Descriptions of hackberry "witches'-brooms" appear in several papers on eriophyids; Keifer (1957) illustrates this condition on *Celtis occidentalis*. Injury, gall formation, and host plant distortion in Kansas are indistinguishable from the same condtion described in the papers mentioned above.

Many species of eriophyids do not cause noticeable damage to the host plant, and a careful examination of leaf surfaces is necessary to collect these species. The mites are more commonly found on the lower surfaces on and along the edges of veins, frequently with their mouthparts inserted in a vein in the feeding position. When populations are low and no plant injury attracts attention, there is still a clue that may indicate infestation. Cast skins, which look like small white streaks a bit shorter and narrower than the mites that left them, can be found scattered and in patches on the leaf surfaces.

Grasses are difficult to examine for mites because the margins of the blades roll inward and longitudinal furrows may be almost closed. Frequently, mites are most abundant at the bases of the leaves, around and under the ligules. A special plastic stage for a dissecting microscope was found to be very useful in examining grasses for mites. This stage was made of transparent plastic one-fourth of an inch thick, with a ridge of plastic one-half inch high and about two inches long cemented on it. The ridge of plastic was located so that it would extend from the bottom to the top of the visual field. A blade of grass can be drawn across this ridge and thus opened up for examination and at the same time kept in focus on top of the ridge. The plant material can be held with one hand while mites are removed with the other.

The appearance of living mites is rather variable and should be noted in making collections. They may be amber or whitish, opaque or transparent, or even chalky white if waxy secretions are present. Some species are dull shades of red and orange. The setae, especially the dorsal ones, may be black and conspicuous in living specimens although not so in cleared, mounted material.

Preserving: Collected eriophyids may be preserved in several ways. The following methods are more commonly used:

1. Heavily infested leaves or plant parts are wrapped carefully in soft tissue, put into envelopes, and allowed to dry. The dry, mummified specimens removed from such material make excellent mounts which may be superior to those made with fresh or living mites. Such dry materials should be stored in insect-proof containers and fumigated periodically.

2. Seventy percent alcohol may be used to preserve bits of twigs or leaves that are infested with mites. There are objections to this method, for usually eriophyids are difficult to handle in a liquid such as alcohol; the alcohol extracts plant pigments, becomes dark in color, and mites are often difficult to clear. Alcoholic material is not to be considered completely useless, however.
3. Keifer (correspondence) recommends a mixture consisting of 75% water, 15% alcohol, and 10% glycerin for buds and twigs. This is especially good for material that is to be sent through the mail; dry material is easily damaged.

Mounting: Making slide preparations of eriophyids is basically similar to the mounting of other kinds of mites in that they must be cleared and expanded to normal shape, then put on a slide. Since some extremely minute structures must be seen to recognize species of eriophyids, a little staining is helpful.

I have found *minuten nadeln*, pyrex depression slides, and small stender dishes to be useful equipment. The small stainless steel needles are easily mounted in glass handles by heating a solid, soft glass rod red-hot, quickly pressing in the needle and then pulling it out slightly to give a more desirable shape to the molten glass. The tip of the needle is then bent to form a tiny "foot" at the apex. Flattening of the needle to make it more like a tiny spatula also works well but is not always desirable, for such a needle picks up more medium as mites are transferred from one container to another. It is usually better to transfer as little medium as possible in handling specimens.

Recovery of collected mites varies with the methods of preservation. If the materials are in liquid, mites must be transferred individually by needles into the clearing solution. Galls or bits of heavily infested material are not difficult to break off and add to the clearing solution. If such material is not available, then mites suspended in the liquid can be pipetted into small dishes, depression slides, or glass slides and then transferred by needles into the clearing solution. Dry, mummified mites can be handled similarly by putting damage plant parts directly into the clearing solution. If the infestation is heavy, this is a workable system, even when damage to the host is not apparent. Bits of leaves, buds, or blades of grass will usually yield needed specimens. Mites can also be picked up with a needle from the dry leaf and put directly into clearing solution. When specimens are abundant, leaves or blades of grass can be tapped or shaken over a small black plate which then can be examined with the dissecting microscope. Such a plate is especially good for dry grass if specimens are fairly abundant. This saves much time in recovering specimens since they are, in most instances, about the same color as the host plant and, therefore, difficult to see.

Preparation of slides, using Keifer's (1954) solutions, requires considerable handling of mites, transferring them from one solution to another. This is not a difficult procedure, since the media do not harden rapidly. The media may, however, become thickened and sticky if too much time is taken in transferring. If this happens, a drop of fresh medium can be added to the slide to keep the medium soft. After oven drying the final mount at about forty degrees centigrade, an immediate ringing of the slide with clear lacquer is advisable. Gentle heat to spread the medium and dry the slides is recommended. A ring helps to hold the coverslip in place since even the final medium will soften under conditions of high humidity; excessive drying and crystallization are also less likely to occur in ringed mounts.

I have used a slight modification of Keifer's system. The intermediate and final media of Keifer (1954) are still used, but Nesbitt's (1945) solution is substituted for the first solution. Nesbitt's works quickly and very well in preparing dry and fresh material, but specimens must be checked frequently. It may be necessary to dilute Nesbitt's solution, because setae and featherclaws may come off if the mites are left in the undiluted solution too long. The mites should then be placed in Keifer's intermediate solution and left for a day or so before transferring them to the final mount. The intermediate medium is a good study and drawing medium because of its optical properties and mites can be rolled into position easily. This intermediate medium will not harm the specimens, even if they remain in it for long periods of time.

Viscosity and temperature of the medium are both important in making eriophyid slides. If the medium is too cold it becomes too viscous for convenient use. As mounting media age they become more viscous, but a drop or two of water added occasionally will remedy this.

Many of the problems that arise in making slides of eriophyids must be solved by the individual as they occur. The proper amount of iodine needed for staining varies from an almost saturated solution (for observation under an ordinary transmitted-light microscope) to a lightly tinted solution (if a phase microscope is to be used). Keifer (1954) gives formulae and suggested amounts of iodine in his mounting media. (See appendix for formulae of Keifer and Nesbitt.)

The following suggestions are made for placement of eriophyids on the slide: Push the mites into a compact group, if several mites are to be put on a single slide. The specimens will stay fairly close together as the coverslip settles. Orientation of a single specimen is much easier if two small needles are used to rotate or press gently on the coverslip. Forceps or needles may also be used to tap the coverslip very lightly as it settles in order to keep the mites near the center of the coverslip. Most of this is routine, and practice is very important to produce good mounts.

Mite mounting media such as Hoyer's or the polyvinyl alcohol media usually render eriophyids too clear. Occasionally eriophyids will not clear enough in PVA-L-P medium. Beer (1954) explains the formula and preparation of Hoyer's and PVA-L-P media. Actually, mounts in PVA-L-P are sometimes rather good but, when mounted from life, require a long time to clear and still do not compare favorably with the media of Keifer.

A prepared stain mountant, CMC-10S, seems to be very good and specimens can be transferred directly from Nesbitt's clearing solution to this medium. CMC-10S can be purchased from Turtox. a biological supply house. The staining properties of this medium are good, and if specimens are not cleared adequately they may become too darkly stained for phase microscopy. CMC-10S has not been in use long enough to determine the condition of specimens after a number of years. The prospects for using this mountant seem very good.

Measurements: The morphological structures measured and used in the descriptions are figured and labeled in Plate 1, which presents a general view of eriophyid morphology. In this study measurements were made as follows: *length*—caudal end to anterior tip of shield; *width*—at widest point, usually the posterior region of the shield; *leg length*—all segments included, but featherclaw not included; *rostrum*—visible portion; *dorsal estae*—entire length, and distance apart at bases being the distance between centers of the dorsal tubercles. Measurements should be used for comparative studies since some of the smaller species have been drawn to appear about as large as the largest species.

Drawings and measurements have been made using a phase contrast microscope and lightly iodine-stained specimens. The measurements of setae and rostrum cannot be taken as exact but should give some idea of size in comparison with rostra and setae of different species. Variations in these measurements may be due to the amount of staining, retraction (of rostrum), optical system used, and visual acuity of the observer, as well as actual variation that exists in the species.

SYSTEMATIC RELATIONSHIPS

Much more work must be done before definite relationships of eriophyids to other groups of mites can be established. There is a superficial resemblance to other elongate, annulate mites such as democids, nematalycids, and some of the tenuipalpids. The feeding apparatus and phytophagous habit seem to put eriophyids, tetranychids, and the tenuipalpids closer to each other phylogenetically than to other groups. The chelicerae of tenuipalpids and tetranychids are long, slender, needlelike, and protrusible with U-shaped bases. A large, protrusible, basal lobe, the stylophore, is also present in members of these two families. Eriophyids do not possess a stylophore but do have needlelike chelicerae that can be moved slightly back and forth. In the Tenuipalpidae, *Phytoptipalpus*, Tragardh has lost the fourth pair of legs. Tenuipalpids may also have elongate bodies similar to eriophyids. Annulations or markings very much like annulations can be found in all three families. Baker (1952) states that the Eriophyidae, Tetranychidae, and Tenuipalpidae have enough in common to indicate a common ancestry, but it would seem to the present author that such a relationship is a very distant one.

The anterior and transverse arrangement of genital plates is a unique characteristic of the Eriophyidae. Tenuipalpids have a transverse genital opening but it is posterior in position. Two pairs of anteriorly located legs with featherclaws are also found only in the eriophyids. A group so morphologically distinct suggests antiquity and long separation from other groups which may have had an ancestral form in common with the Eriophyoidea.

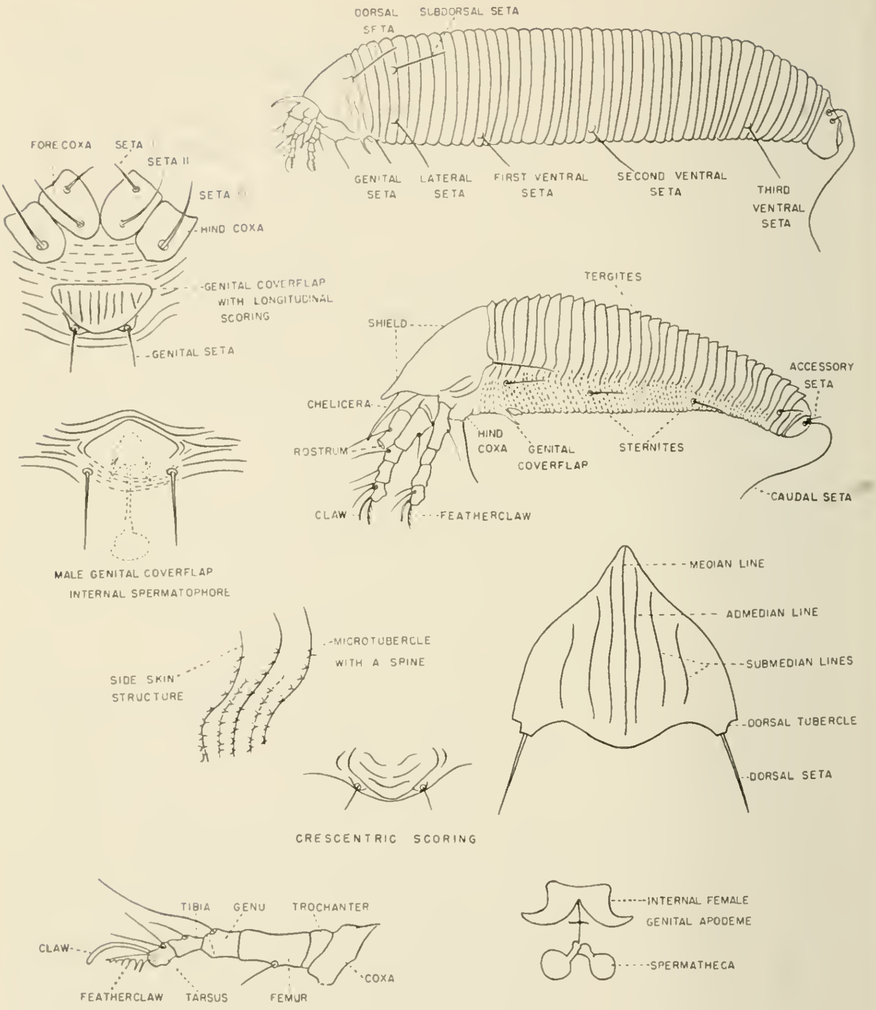


Plate 1

Wide distribution also seems to indicate an old group that has had time to spread to all parts of the world. There is still no evidence concerning the fate of the posterior two pairs of legs in eriophyids. Additional information on circumstances leading to the loss of the fourth pair of legs of *Phytoptipalpus* may help in solving the riddle of loss of two pairs of legs in eriophyids, and at such time the relationships of the latter may be much clearer.

Resemblance to other ipomorphic groups of mites, such as demodicids, is little more than similarity in shape; the similarity clearly results from parallelisms rather than phyletic relationship. Eriophyids still seem to stand as a single group, the Tetrapodili; two pairs of legs, a wormlike annulate body, proximal genitalia, and an oral stylet comprise the distinguishing characteristics of the family.

MORPHOLOGY

Hassan (1928) and Keifer (1959) provided the major papers dealing with eriophyid morphology. Hassan gave a rather general account, while Keifer's paper is a very detailed study of the gnathosoma of eriophyids.

Eriophyid morphology is discussed briefly here for convenient reference. For more detailed accounts the above mentioned papers should be consulted.

Because of its unusual body design, including the absence of posterior pairs of legs, an eriophyid's body only can be divided into two regions, the gnathosoma and the idiosoma. The gnathosoma includes the mouth opening and the adjacent appendages which form the rostrum. Several internal structures should also be considered as parts of the gnathosoma. These internal structures, according to Keifer (1959), are the pharyngeal pump, hinge, motivator, and pump brace. The gnathosoma consists chieflly of the beak or rostrum. The rostrum is made up of the palpi that form an anterior groove in which lie the following needlelike mouthparts: chelicerae, auxiliary stylets, oral stylet, cheliceral guide, and cheliceral sheath. The use of the needlelike mouthparts, which can not be retracted or extended, is described very clearly by Keifer (1959), who says that the terminal segments of the palpi telescope or fold back and make it possible for the sharp mouthparts to penetrate plant tissue.

The body of an eriophyid is elongate, wormlike, and annulate. Two pairs of legs arise anteriorly on the body. Ventrally, immediately posterior to the legs but still far anteriorly, are situated the genitalic structures of eriophyids. The genital plates are transversely arranged, somewhat similar to those of tenuipalpids, but in tenuipalpids the genitalia are posteriorly located. Baker's (1952) presumption that this difference is not as significant as one might suppose seems quite acceptable. There probably has been a coalescing of posterior body segments in the tenuipalpids, resulting in the location of the genitalia on the extreme end rather than in a more or less normal position, according to Baker.

Externally the genitalia consist of a coverflap arched posteriorly in females and anteriorly in males. Internally, female eriophyids have what Keifer calls the anterior genital apodeme. Just posterior to this apodeme are glandlike structures. The function of these glandlike structures or small sacks has not been determined definitely; it is here suggested that they are spermathecae. Males also show internal genital structures, as has been noted by Hall (1958, plate 1) among others, but they are much more difficult to see and do not appear consistently. Since males are not known for many species, little taxonomic use has been made of male characters. It may be that in some species these internal structures of males may prove useful, but at present little is known about them.

The leg of an eriophyid has a coxa, trochanter, femur, genu, tibia, and tarsus terminated by claw and featherclaw. The featherclaw with lateral rays is extremely useful as a taxonomic character at the species level; its axis may be entire or divided. The featherclaw is the empodium. Sometimes legs show considerable reduction and even loss of a segment. The subfamily Nothopodinae lacks a distinct tibia and is distinguished on this basis, plus the fusion of the anterior coxae with the suboral plate.

Setae of eriophyids are not as variable as they are in many other groups of mites, where they are often highly specialized and of many forms. It almost appears that eriophyids are so well established on the host, with overwintering and dispersal techniques solved, that they have no need for the special tactile and chemoreceptive structures present in other mites. However, the setae are sufficiently variable in size, location and number to have some taxonomic significance.

GEOGRAPHICAL DISTRIBUTION AND HOST AFFINITY

Although many parts of the world remain uninvestigated for eriophyids, they are undoubtedly present in all regions where higher plants exist. Most of the species described occur in Europe or California. A few species have been described from Java and Puerto Rico, and some economically important species are under observation in Russia and Poland.

The variety of host plants affected, the variations in life histories, the tremendous numbers of individuals, and extremely small size suited for several methods of dispersal all contribute toward world-wide distribution of this group of mites.

Some species are known to be cosmopolitan. Host requirements apparently determine ranges of other species. As preservation of specimens improves and type specimens become available, many other species may be found to be cosmopolitan. Because of the ephemeral nature of many preparations, students of eriophyids have been seriously hampered by the inadequacy or lack of type material upon which earlier descriptions are based.

Obviously eriophyids occur throughout the United States. In my personal collection are specimens from Michigan, Florida, Texas, Colorado, Nebraska, Missouri, Oklahoma, Alabama, Kansas, and California. However, in all states mentioned except California and Kansas, collection has not been intensive.

Just how host specific eriophyids are is difficult to say. Keifer (1952) states that their host relations are intimate and species nearly always show a high degree of specificity. Many species do occur on two or more closely related plant species. For example, Keifer (1952) reports that *Aceria brachytarsus* (Keifer) forms purse galls on both species of black walnuts native to California but will not attack the imported English walnut now growing in the same area. Some have host ranges extending through related plant genera; however, only *Diptacus gigantorhynchus* (Nalepa) crosses plant family lines in California, according to Keifer. It occurs on *Prunus* sp. (Rosaceae) and *Vitis californicus* Bentham (Vitaceae). *Aceria tulipae* (Keifer) is known at present from 13 different hosts in two families of plants, Gramineae and Liliaceae, both of which are monocotyledons.

Some species are known only from a single host species, but the host may be attacked by several species of eriophyids. Occasionally a single host will harbor two closely related and morphologically similar species, one of which causes conspicuous injury to, or galls on, the host while the other does not.

Host association was very important in the early history of eriophyid identification, but in recent years the discovery of more species has pointed out the limitations of relying solely on this method. It is still an important guide to identification, however, especially if the type of injury to the host is also known.

LIFE HISTORY

The life history of an eriophyid may be a simple type of development, or it may be rather complex if a deuterogynous species is concerned. A few detailed life history studies have been made by various workers. The findings of these studies, together with a general account of each type of life history, are given below.

Techniques for handling and observing living eriophyids are discussed by Baker (1939), Keifer (1941, 1942), and Rosario (1958).

SIMPLE LIFE HISTORIES

A simple life history cycle requires ten days to two weeks for completion. After hatching, two nymphal instars must be completed before the sexually mature adult state is reached. This refers to females only; it is not clear just what route males follow in the simple life history. For many species males are not known. Thus, the simple life history consists of egg, two nymphal stages, and the adult stage.

Vasates cercidis on *Cercis canadensis* L. was observed during this study and yielded the following information. In the latter part of October eriophyids were observed in abundance on the lower sides of leaves. After two nights of light freezing temperatures, mites were still abundant on the lower surfaces of the leaves. Mites were present on leaves until leaf drop and many perished with the leaves after they fell to the ground. Leaves that were not too dry, picked up from the ground, still had a few living mites on them in early November. On November 18, branches of last year's growth were brought into the laboratory and mites were found in considerable numbers under the buds (between the bud and the branch). Several mites observed in these areas were dead, but many looked normal and were alive. The mites were not active and were concentrated in the grooves and cracks between the base of the bud and the branch. On January 13, twigs were brought into the laboratory and examined by chipping material away from the outer basal areas of the bud. Hundreds of mites could be found in these areas. Most of them were light orange in color and somewhat flattened. The majority of these mites were dead and completely desiccated. In some areas such as better protected pockets or cracks, clumps of six or eight mites could be found. These mites were not dried up but appeared normal and became active after warming in the laboratory. The live mites were light amber in color and moved about very little, usually remaining in one spot and making slight movements.

As time progressed, fewer and fewer living mites could be found so that by March 16, areas that previously had eight or ten living mites now had only one or two. At this time the buds were just beginning to open. On March 31, mites were still very scarce at the bases of the buds. Weather was still cool with freezing but not severe temperatures. By April 14, the redbud trees were beginning to bloom. Eriophyids at this time were still scarce and were found at the bases of the flowers, on the receptables. Mites continued their emergence from hiding and fed especially on the basal parts of the blooms. No mites could be found at the bases of buds at this time, but they were rather numerous at the flower bases. No eggs were present as yet; adults were opaque but not chalky white. By April 26, the mites had moved to the young leaves, assembling around the distal ends of the petioles and on the undersides in angles of the veins. They were also present on new stems. Males were present but not common; two immatures were noted on one young leaf. It is not clear whether these males and immatures came from unseen eggs laid in spring or whether they had overwintered. Observations on April 30 showed scattered eggs laid on the lower surfaces of leaves along the veins. When first laid, eggs are slightly opaque with a low gloss. On May 1 the eggs looked much the same; some eggs were beginning to turn a milky white and two blackish, dark streaks could be seen inside. These dark streaks were the heavy black setae of the shield, characteristic of this species. On May 3, populations of mites were becoming established at the bases of leaves. Six to ten mites were noted at this time on some of the leaves; eggs and nymphs were observed about the base of the leaf blade and in the angles of the veins. Populations were still very low with only a dozen or so mites on some of the leaves. By the middle of May the new generation, including nymphs and adults, was established and it seemed that about every month a new generation was produced. There is apparently considerable overlapping of generations, but it seems that at least three generations are produced in the summer months. Late in the summer many mites become sequestered in protected areas where buds are produced and, as already noted, many remain on and perish with the leaves.

My observations are in harmony with other life history studies of eriophyids. There is the probability that a greater percentage of overwintering forms die and that more generations may be produced by this Kansas species than by the other species studied. Summer temperatures in Kansas are high, frequently in excess of 100°F which may account for the several summer generations.

One other Kansas species, *Aceria slykhuisi* Hall, was observed rather closely in this study. The host of this species is *Buchloe dactyloides* (Nuttall). Hall (1958) gives a brief account of relationships to the host and to other mites (tarsonemids) that commonly occur on the same host. To observe the mites, samples of grass were obtained from Fort Hays Experiment Station where outdoor test plots of *Buchloe dactyloides* were maintained. In the summer of 1954 four sprouts of this grass were placed in seedling flats in the greenhouse. These samples of grass seemed free of eriophyids; there was no evidence of mite damage to the host. Most of this grass died down, but some was maintained through the winter. As runners were put out in April of 1955, "witches'-broom" symptoms began to appear and eriophyids were numerous in these deformities. More abnormal tufts of grass formed and eriophyids became exceedingly numerous in these tufts. Predatory phytoseiid mites were occasionally seen in these cultures. By May 25, populations of tarsonemids, *Steneotarsonemus spirifex* (Marchal), were seen in the "brooms," coexisting with the eriophyids. The tarsonemids became numerous but just what effect they had on growth of the host or on the eriophyids is not clear. It is certain that the "witches'-brooms" and the eriophyids appeared before the tarsonemids and it thus is apparent that the plant growth deformity is caused by eriophyid feeding.

Vasates dimidiatus, described in this paper from *Populus deltoides* Marshall, was observed from egg to adult stage. Eggs hatched into nymphs in approximately eight days. The egg to adult period was about two weeks. (For relation to host see the description of this species.)

In 1957, Minder, a Russian worker, carefully studied the life history of *Eriophyes pyri* (Pagenstecher) which infested pear orchards and caused as much as 95 percent crop loss. The more significant points in his study are given below:

The egg to adult period was 20 to 25 days. Only two generations per year were noted and overwintering began in June. Females and a few nymphs overwintered in the buds. Males were not observed in the first generation and constituted only 0.5 percent of the second generation.

Minder also mentions the manner of gall formation by the host plant in response to the feeding of *Eriophyes pyri* (Pagenstecher). Osmotic pressure of fluids in the leaves is mentioned by Minder as a possible factor in plant resistance to mite attack.

COMPLEX LIFE HISTORIES

The complex life history was not understood until Keifer (1942) discovered two types of females in the buckeye rust mite, *Oxypleurites aesculifoliae* Keifer. Later in his generalized report of the life cycle of deuterogynous species, Keifer mentioned the existence of one female, the protogyne, which is associated with males; the other structurally different female is the deutogyne which is specialized for hibernation or aestivation and is not associated with males. The protogyne or primary form is morphologically similar to the male and occurs more commonly on the leaves of the host plant. Keifer states that deutogynes appear in response to leaf maturation or to the coming of lower fall temperatures. Deutogynes do not reproduce in the year that they develop; they feed on the leaves, then withdraw to bark crevices or lateral buds where they overwinter. Deutogynes will enter diapause after feeding regardless of the season. Overwintered deutogynes come out of hibernation in the spring and lay eggs on the new leaves; these hatch into males and protogynes. The primary females (protogynes) then lay eggs which produce primary or both primary and secondary females (deutogynes), as well as males.

In his studies of the buckeye rust mite in Marin County, California, Keifer found that deutogynes became active in late winter, left their hibernating quarters on twigs, and when buds swelled in February, penetrated beneath the outer scales. There they fed on the green tissues of the inner scales. With the development of the early spring leaves, the deutogynes laid eggs which hatched into nymphs, producing primary mites of both sexes on the leaves. The primaries soon began active reproduction of additional primary mites. Beginning the last of April or early May, Keifer found new deutogynes appearing among the primary types. When fully fed, these deutogynes traveled down the stem six inches or more. There they crawled into crevices or other shelters on the previous season's wood. Thus deutogynes appeared to abandon the leaves during June and July. The primary females remained on the leaves and green tissue and perished with it, although reproduction had almost ceased by early July.

Putman (1939), working with another deuterogynous species, *Vasates fockeui* (Nalepa and Trouessart) suggested that hardening of foliage may have something to do with the production of overwintering forms. Keifer does not disagree with this idea. However, it is Putman's belief that overwintering females may be fertilized before hibernation. This idea came from his observation that unfertilized protogynes produced only males while overwintering females (deutogynes) produced both males and females. Thus, we see that in *V. fockeui*, protogynes are arrhenotokous. Since there is little proof available on fertilization or lack of it one can not be certain that deuterotoky exists in eriophyids. Burditt (1963) indicates in *Phyllocoptruta oleivora* (Ashmead) and *Aculus pelekassi* Keifer that fertilized females produced male and female offspring and unfertilized females produced only males. Here then are two more examples of arrhenotoky.

Shevtshenko (1957) has given an interesting account of the life history of another deuterogynous species *Eriophyes laevis* (Nalepa), summarized below.

The egg to adult period was 23 to 25 days. Two and sometimes three generations of protogynes were produced by *Eriophyes laevis*. Deutogynes appeared in July with maximum number in August. The deutogynes immediately left galls and went to the overwintering sites. Eggs were laid by deutogynes only after overwintering, and these eggs produced female protogynes. Protogyne eggs developed into protogynes, deutogynes, and males. Males appeared early in the second generation. Shevtshenko did not observe mating but suggested parthenogenesis in the deutogynes and spermatophore formation in protogynes.

There is some recent evidence of ovoviviparity in three species. Shevtshenko (1961) mentions this in *Eriophyes laevis* (Nalepa); his drawing shows a female with two nymphs inside the body. The two eggs from which the nymphs hatched are also shown inside the female's body with the two nymphs. He does not indicate whether this ovovivipaparous female was a protogyne or a deutogyne.

I have observed the same thing in *Vasates quadripedes* Shimer. A protogyne female on a prepared slide shows two nymphs and two empty eggs inside the body (plate 27). This method would obviously reduce the number of young produced by a single female and may explain why the ovoviviparous habit is not widespread in eriophyids. Fewer offspring would reduce the possibility of survival and production of another generation.

Burditt, Reed, and Crittenden (1963) came across another example of ovoviviparity in *Phyllocoptruta oleivora* (Ashmead). Although this is not reported in their paper, D. K. Reed sent me photographs of three female specimens with a single nymph inside the body of each female.

The senior author Burditt (1963) reports a single observation of copulation in eriophyids. During copulation, the female almost completely covered the male, according to Burditt. This is the complete and, to my knowledge, the only record of mating in eriophyids. D. K. Reed indicates (correspondence) that round-the-clock observations are being made in the USDA laboratories at Orlando, Florida. I have no information concerning the results of this work.

In general, the life history studies by Baker (1939), Putman (1939), Keifer (1942), Minder (1957), and Shevtshenko (1957) are in agreement. Some differences do exist in that most authors report that deutogyne eggs produce only females, but Putman found that deutogyne eggs produced males and protogynes and unfertilized protogynes produced only males. There may well be specific or generic differences in such matters.

A number of points (for example, mating and sex determination) are still not explained. The exact role of eriophyid males is still uncertain. Mating has been reported from only one observation (Burditt, 1963). Males are present in populations of many species, sometimes abundant, sometimes scarce or absent. Deutogynes, the overwintering females, in most cases produce only female protogynes, but in at least one case (Putman, 1939) deutogynes seem to produce both males and female protogynes. Shevtshenko (1957) suggested spermatophore formation and E. W. Baker (correspondence) also speculates that males deposit spermatophores and females pick them up. This unsolved point in eriophyid life histories is a challenging problem which merits immediate attention. I feel that spermatophore formation and transfer is the probable mechanism of fertilization in eriophyids.

I have noted occasionally in males some internal genital structures that occupy the same relative position as female genital apodemes and spermathecae. The function of these structures in the male is not known. From the structure of these internal genital parts in the male (see *Aceria slykhuisi* Hall, plate 13, and *Abacarus sporoboli* (K.), plate 2), it seems that the anterior end is modified for attachment or for holding. Since mating is still not fully described in eriophyids, I judge that these anterior recurved or barbed structures may be part of a spermatophore and perhaps aid in removal of the spermatophore from the body of the male. These internal male genital structures are posteriorly modified into glandlike bodies that may be parts of the supposed spermatophore.

It is easy to understand why, in some instances, two types of females belonging to the same species were described as two different species. For example, in the life history of *Oxypleurites aesculifoliae* Keifer the deutogynes were described as *Phyllocoptes aesculifoliae* Keifer in 1938. Two months later the primary type was named *Oxypleurites neocarinatus* Keifer. A great deal of confusion in eriophyid life histories was cleared up by the recognition of deuterogyny. Here then is another example of research of fundamental nature on non-economic species leading to the solution of a problem in an economic species. In this case, the pear leaf rust mite, *Epitrimerus pirifoliae*

Keifer, was better understood with the discovery of two kinds of females in *Oxypleurites aesculifoliae* K.

BEHAVIOR

There is considerable variation in the behavior of the species of eriophyids. Virus transmission work, mite dispersal studies, and life history observations have emphasized similarities and differences.

Dispersal of eriophyids is discussed here because their behavior in response to growth or death of the host determines whether they stay on that host or take a position that will cause them to be blown or carried to another area and possibly to an uninfested host. Three methods of eriophyid dispersal are known: wind, insect, and man (by budding). Wind is undoubtedly the chief means of dispersal; dispersal by insects is probably second in importance. The transfer of specimens from one host to another by man, in budding or grafting plants, is of little importance and would involve only a few economically important species.

Eriophyids that live on grasses, especially wheat, do not have survival sites such as buds and bark, and they must find other survival sites. This usually means leaving the host. Gibson and Painter (1956) give evidence that wheat kernels infested with eriophyids are the source of mites infesting new wheat seedlings produced by such kernels. As infested kernels of wheat drop to the ground and sprout the mites frequently move directly from the kernels to the new wheat seedlings. Therefore, it seems that mite-infested kernels are important survival and overwintering sites. Gibson and Painter (1957) state for *Aceria tulipae* that, as plants begin to die, the mites migrate upwards with thousands concentrating on the tips of leaves. There they crawl upon one another, often forming chains of several individuals connected by their anal suckers. In the greenhouse many of these chains separated from the mass and fell to the soil below. Mites in the field are readily air-borne and undoubtedly this is the primary means of dissemination. Air-borne specimens of *Aceria tulipae* have been collected 150 feet above ground and one to two miles from the nearest wheat fields where the species would normally be found (Pady, 1955).

A kind of behavior seen in nearly all species of eriophyids consists of holding the body perpendicular to the leaf surface and adhering to the surface by the anal sucker or by sticky secretions. The posterior end of an eriophyid mite is bilobed and perhaps can be used in a pinching action to cling to the leaf surface. In this perpendicular position they are more likely to be blown from the leaf surface. This also seems an advantageous position for attachment to insects as they pass by.

Dispersal by insects was also noted by Gibson and Painter (1957) who observed mites attached to the body of an aphid. This method of dispersal probably occurs frequently in the field and could be important in getting mites to volunteer wheat in fringe areas of a wheat field. Spots of volunteer wheat that are not cut and plowed may be important survival sites. Attachment to insects may be the chief means of reaching alternative hosts since the aphid may go directly to another host.

Movements of mites on woody plants vary with the seasons. As new growth appears in the spring, mites come out of overwintering sites and move upward. This upward movement is negative to gravity according to Shevtshenko (see Life History section). The season when the buds swell seems to be the time that upward migrations begin and, as this is synchronized with rising temperature, it may trigger the movement. In the fall, mites on trees and shrubs move downward or toward the last year's growth to sequester themselves in cracks of bark or in buds for overwintering.

Eriophyids can move about rather quickly even though they are extremely small. Minder (see Life History section) gives the rate of movement as a maximum of 10 to 15 mm per minute. This indicates that they can move easily to new growth and spread to all parts of a single tree or shrub without the aid of special dispersal methods such as wind or insects.

Response to light may be involved in movement. Negative reaction to light has been reported by Rosario and Sill (1958) who were able to transfer *A. tulipae* from one leaf to another by directing a beam of light at them. A flashlight (cold light) seemed to work as well as a 50-watt incandescent light. Movement of the same species to leaf tips, as described above, shows that at certain times or under certain circumstances other factors counterbalance the response to light.

Ordinarily eggs of eriophyids are scattered on the leaf surface or along veins. On grasses, eggs may be placed in the longitudinal furrows and more often at the base of the leaf under and near the ligule. Eggs are apparently sticky and remain attached where they are placed, even when laid on the tips of plant hairs (see *Rhyncaphytoptus boczeki* discussed below). Nymphs feed and then attach themselves to the leaf surface; they appear to be stuck to the surface and as they molt, the cast skin, appearing as a white streak, is left at the place of attachment.

Little is known about the manner of excretion in this group of mites. There is no evidence of excrement, even inside a small gall where hundreds of mites are living. Since plant juices serve as food for eriophyids, excrement may be excess fluid passed on through the digestive tract and this may be absorbed by the plant. If the excrement is fluid, this would be very difficult to see on the leaf surface.

Due to their small size and habit of living in protected areas such as galls, cracks, and buds, eriophyids have little need for special defense mechanisms. Most of the galls are lined with an erineum and the opening into them is so completely blocked by these plant hairs that any predator larger than an eriophyid would have difficulty entering the gall.

I observed that *Rhyncaphytoptus boczeki* has a method of oviposition and molting which seems to give some protection from predators. This species occurs on *Celtis occidentalis* L. Eggs are laid singly and in groups of two or three at the tips and near the tips of plant hairs, completely removed from the leaf surface. After hatching and feeding, most of the immatures crawl back up the plant hairs, attach their posterior ends to the hair and extend the rest of their bodies out into space parallel to the leaf surface but well above it. Some individuals attach parallel to the plant hairs or along them rather than extending their bodies. Molting occurs in these unusual positions. I have observed possible predators (e.g. phytoseiids) walking about on the leaf surface, passing under these eggs and molting forms, apparently without sensing their presence.

MORPHOLOGICAL AND BEHAVIORAL ADAPTATIONS

Morphologically eriophyids seem extremely well adapted to their environment. Size and body shape are such that they can exist in abundance in protected spaces of such small size that almost all other arthropods are excluded. Good protection and easy dispersal by wind to other host plants seem to make this a highly successful family. Undoubtedly protection and good dispersal, which are important factors of survival in any species, are possible because of the small size.

It is interesting to note that eriophyids living in galls of various sorts and those living as vagrants on leaf surfaces are different in appearance. Those living in galls have bodies that are evenly contoured and lack the bizzare undulations, ridges, and folds seen in many vagrant species. I believe that vagrant forms with these folds and undulations have evolved into the evenly contoured types that live in galls. The body folds and ridges would certainly be disadvantageous to them in small galls or tightly rolled leaf margins. With spacial restrictions of the microhabitat imposed by the confining walls of such galls, populations get to be so large that the mites appear to be literally packed into such areas. Complex body form would be a serious disadvantage under these circumstances.

It is also possible that gall forms could be evolving into the free living form, but this seems less likely because the formation of galls must have come about after free living mites became established on plants. Mechanisms of producing galls probably evolved in vagrant species, and this was followed by morphological adaptation to this microhabitat.

Keifer (1966) discusses the subfamily Aberoptinae which contains species capable of mechanically damaging the host plant. Most damage caused by eriophyids is biochemical in nature but at least two species seem capable of causing mechanical damage. *Aberoptus samoae* K. has spatulate foretibiae and *Cisaberoptus kenyae* K. has a rostrum that is quite distinct. These structures seem well adapted for the habit of burrowing under the leaf surface. *C. kenyae* K. does just this and it is not known exactly what *A. samoae* K. does, but it certainly seems equipped to cause mechanical damage.

The possession of only two pairs of legs is perhaps of some benefit to eriophyids. They can move about easily among plant hairs and in furrows with the two pairs of anterior legs dragging the elongate body. The legs are situated so that movement is accomplished by reaching forward and pulling, without lateral extension or movement of the legs which would hamper movement on pubescent or furrowed leaf surfaces. The loss of legs is perhaps another adaptation which has made it possible for eriophyids to occupy galls.

Body setae are directed posteriorly which would also be an advantage in forward movement among plant hairs or furrows. Occasionally shield setae are directed anteriorly or medially, but these are invariably shorter, do not extend much beyond the body limits, and would therefore not seem to interfere seriously with movement.

Eriophyids lack special sensory setae. The abundance of food and easy dispersal seem to reduce the need for them. Featherclaws of eriophyids are the only setae that show much modification, and their exact function is not known. Judging from the appearance of featherclaws, I would say that they could be tactile or adhesive in function. Featherclaws are possibly useful in locating feeding sites such as veins.

Behavioral adaptations of eriophyids are directed along three lines: overwintering, protection, and dispersal.

Overwintering adaptations apparently have been made in response to seasonal (or climatic) changes. Regardless of the exact stimulus, a method has evolved of surviving the season of plant dormancy. In some species, females and a few nymphs overwinter in small protective cracks or in buds and start new populations in the spring as new growth begins. A higher degree of specialization is seen in deuterogynous species which feed and immediately go into overwintering sites, remaining inactive until next spring when they lay eggs. Deuterogyny would seem to be more beneficial to species living on deciduous trees or plants with a short growing season. A new generation is assured by the overwintering deutogynes even though leaf drop occurs with many individuals dying on the leaves. Actually there are no deuterogynous species known from gymnosperms or broadleaf evergreens. Species living on these plants are probably not challenged by the problem of overwintering because of the usually evergreen foliage. No deuterogynous species are known from broadleaf evergreens.

A presumably protective habit of one vagrant species, *Rhyncaphytoptus boczeki*, is the laying of eggs on the apices of plant hairs rather than on the leaf surfaces. Molting also takes place well above the leaf surface where discovery by predators is less likely.

An adaptation that undoubtedly enhances dispersal is the mass migration of eriophyids to the uppermost leaf apices where they form chains of individuals which break off and are readily airborne. Dispersal resulting from attachment to insects is perhaps accidental, but it does seem that the habit of raising and holding their bodies perpendicular to the surface of the leaf with their legs free would make it much easier for eriophyids to attach to insects and be carried to other plants.

Morphological and behavioral adaptations of eriophyids have reached what appears to be a rather stable condition. This could be due to severe pressures of the environment. Slight changes in morphology or behavior would be eliminated from the population quickly and thus would favor the maintenance of a group with uniform characteristics. Such a situation, prevailing over a long period of time, would also explain the obscurity of the ancestry of the group as well as the large number of similar species in a small number of genera.

ECONOMIC IMPORTANCE

Direct damage to fruit and foliage as well as the transmission of virus diseases to host plants by eriophyids emphasize the economic importance of the ubiquitous group. The following are four virus diseases known to be transmitted by eriophyids: current reversion, fig mosaic, peach mosaic, and wheat streak mosaic. Losses due to these diseases are great, and when added to those caused by mites feeding on foliage and fruit, the amount is millions of dollars annually. There are reports of crop losses of pears as high as 95 percent (Minder, 1957) due entirely to eriophyid infestations.

Economic papers on eriophyids are very numerous and a complete account is not given here. Only occasionally are losses estimated in dollars. In Kansas, *Aceria tulipae* (Keifer) is the most important species economically. Kantack and Knutson (1958) cite losses due to wheat streak mosaic vectored by *Aceria tulipae* as $30,000,000 in 1949 and $14,000,000 in 1954. Considering all wheat growing areas, these figures would be increased considerably, at least enough to warrant exhaustive studies of this species to determine the best methods of control. No estimates are available on losses due to other virus diseases carried by eriophyids.

The transmission of peach mosaic virus by *Eriophyes insidiosus* Keifer and Wilson was shown first by Wilson, Jones, and Cochran (1955). This discovery came after some 8,000 tests had been made, using about 150 species of suspected arthropods. About 20 years of research preceded this discovery. Peach mosaic occurs in California, Colorado, Texas, Oklahoma, and Arkansas. The vector was easily collected in all these areas. It is likely that this virus also occurs in Kansas, but it has not yet been reported.

Aceria ficus (Cotte), the vector for fig mosaic common in California, has not been collected in Kansas. No estimates of losses are given for fig mosaic. Currant reversion disease, carried by the currant big bud mite, *Cecidophyes ribis* (Nalepa), is of no importance in Kansas.

Undoubtedly there are other virus diseases vectored by eriophyid mites, and some species, in addition to *Aceria tulipae* (Keifer), may yet be found to have a role in streak mosaic of wheat. Since eriophyids occur on several grasses and many trees and shrubs, often with little or no damage visible on some hosts, they should at least be kept in mind as potential virus transmitters and as a group of potential economic importance.

Control of eriophyids is rather difficult due to the small size and, frequently, the inaccessibility of the mites on the host. Kantack and Knutson (1958) summarize control studies of this mite. The highest degree of control is reported as 90 percent using Shell OS-1808 which has a very low residual action. Another problem in controlling a species such as *Aceria tulipae* (Keifer) is the presence of alternate host grasses adjacent to, as well as remote from, the wheat fields. Good residual acaricides would possibly help to eliminate this problem. At present such acaricides are not available, and even if they were, the new growth would have no protectant.

Fruit trees are about the only woody plants on which control studies have been made. Boyce (1942) and Spencer (1950) indicate that good control of eriophyids on fruit trees can be achieved using various sulfur or sulfur-containing materials. Spraying was found to be more effective than dusting. The time of spray application is very important in that mites are more easily killed when exposed and moving about.

A number of papers dealing with wheat streak mosaic have been produced at Kansas State University. A list of these publications may be obtained by writing H. W. Somsen, Entomologist, U.S.D.A., Entomology Research, Kansas State University, Manhattan, Kansas.

Boczek (1966) published an extensive bibliography of mites affecting plants and stored food products. This is a good source of information for the researcher interested in economically important species.

SYSTEMATIC ACCOUNT

Until recently the family Eriophyidae was divided into eight sub-families. Keifer (1964) pointed out that three distinct structural groups are apparent and proposed three families under the Eriophyoidea. The following family descriptions are taken from Keifer (1964). Generic examples are given in his Eriophyid Studies B-11.

PHYTOPTIDAE Murray 1877

Three or four setae on cephalothoracic shield, the rear pair pointing straight or diagonally forward; a pair of subdorsal abdominal setae a short distance behind shield, present or absent. Rostrum usually large and evenly down-curved, with apical recurved portion of oral stylet shorter than base plus pharyngeal pump. Legs with all segments and with anterolateral spur on tibia present or absent. Female genital coverflap never ribbed; anterior internal apodeme always moderately long; spermathecae short or long-stalked, but with stalks or tubes projecting forward first and then recurved. Habit: gall formers, bud mites, rust mites, or leaf vagrants.

ERIOPHYIDAE Nalepa 1898 (as here restricted)

Body either wormlike or fusiform, often flattened. Two or no setae on cephalothoracic shield; setae when present on shield located from central area to rear margin, pointing in various directions according to type. No subdorsal abdominal setae. Rostrum large or small, either down-curved or projecting straight down; apical portion of oral stylet shorter than base plus pharyngeal pump. Legs usually with all setae and segments, less often with tibia fused to tarsus, never with lateral tibial spur. Female genital coverflap usually with a pattern of ribs; anterior internal apodeme either projecting ahead from base line or short and transverse; spermathecae short-stalked the stalks or tubes either projecting laterally or posteriorly from origin. Habit: gall formers, bud mites, leaf or green stem vagrants, rust mites.

RHYNCAPHYTOPTIDAE Keifer 1961

Body stout or elongate, fusiform and tapering, not flattened. Cephalothoracic shield with two or no setae, when present the setae located near rear shield margin and pointing forward in some degree. No subdorsal abdominal setae. Rostrum always large, usually abruptly bent down from near base, and tapering; apical portion of oral stylet longer than base plus pharyngeal pump. Legs usually with all six segments, or tibia or patella absent; femoral seta and others frequently absent; never with lateral tibial spur. Ribbing on female genital coverflap usually but not always absent; internal apodeme extending forward, broad or acuminate; spermathecae short-stalked, the stalks extending laterally or to rear. Habit: rust mites or leaf vagrants.

KEY TO FAMILIES

1. Three or four shield setae present, rostrum large, evenly curved downward; apical recurved portion of oral stylet shorter than base plus pharyngeal pump .... Phytoptidae

1. Two or no shield setae present; rostrum abruptly bent down from near base, evenly curved downward or extending straight down; apical portion of oral stylet longer or shorter than base plus pharyngeal pump 2
2. Rostrum large abruptly bent down near base; two shield setae, if present, pointing forward in some degree; apical portion of oral stylet longer than base plus pharyngeal pump .... Rhyncaphytoptidae
2. Rostrum large or small, down curved evenly or extended straight down; two shield setae, when present, variable in position and direction; apical portion of oral stylet shorter than base plus pharyngeal pump .... Eriophyidae

KEY TO KANSAS GENERA

1. One or two frontal shield setae present in addition to the usual posterior dorsal setae .... *Phytoptus*
1. Only dorsal setae present or no shield setae present 2
2. Featherclaw divided .... *Apodiptacus*
2. Featherclaw not divided 3
3. Rostrum large, projecting straight down; chelicerae abruptly bent .... *Rhyncaphytoptus*
3. Rostrum not always large; chelicerae evenly curved 4
4. Abdomen bearing three longitudinal wax producing ridges .... *Abacarus*
4. Abdomen having more than three wax producing ridges or such ridges lacking 5
5. Abdomen bearing four longitudinal wax producing ridges .... *Mesalox*
5. Wax producing ridges not present 6
6. Lateral toothlike projections or lobes present on the abdomen .... *Oxypleurites*
6. Lateral toothlike projections or lobes not present on the abdomen 7
7. Shield without setae .... *Cecidophyopsis*
7. Shield bearing two or more setae 8
8. A sublateral groove present on the abdomen .... *Platyphytoptus*
8. Without a sublateral groove on the abdomen 9
9. An anterior shieldlike lobe over the rostrum; tubercles ahead of rear margin of shield, setae directed anteriorly and usually converging .... *Phyllocoptes*
9. An anterior shieldlike lobe over the rostrum present or absent; tubercles on or in front of rear margin of shield, dorsal setae directed caudad if shield lobe is present and variable in position if shield lobe is absent 10
10. An anterior shieldlike lobe over the rostrum; dorsal setae on or

very near rear margin of shield, dorsal seta placed somewhat laterally and diverging caudad or occasionally more medial in position and converging slightly caudad .... *Vasates*

10. Anterior shield like lobe over the rostrum thin, minute, or absent; dorsal setae variable 11

11. Shield setae on rear margin of shield, directed caudad .... *Aceria*

11. Shield setae in front of rear margin of shield, directed anteriorly or posteriorly .... *Eriophyes*

PLATE SYMBOLS

The following symbols refer to the structures in plates 2 to 32.

AP—Anterior genital apodeme. APl—Internal female genitalia. DA—Dorsal view of anterior section of shield. ES—Side skin structure. EV—Ventral skin structure. F—Featherclaw. Fl—Featherclaw and tarsus. FD—Featherclaw of deutogyne. GF—Female genitalia, ventral view. GFl—Female genitalia and coxae from below. GM—Male genitali.a GMl—Male genitalia and coxae. GMS—Male genitalia, spermatophore. L—Left legs. Ll—Left anterior leg. LT—Tartus and associated structures. R—Rostrum. S—Side view of adult mite. SA—Side view of anterior section of mite. SP—Side view of posterior section of mite. SPl—Side view of protogyne. STH—Spermatheca. V—Ventral view of mite.

LIST OF PLATES

1. External Anatomy and Genitalia. 2. *Abacarus sporoboli* (Keifer). 3. *Aceria parulmi* (Keifer). 4. *Aceria cactorum* (Keifer), after Keifer. 5. *Aceria caryae* (Keifer), after Keifer. 6. *Aceria celtis* (Kendall). 7. *Aceria cynodonis* Wilson. 8. *Aceria erineus* (Nalepa), after Keifer. 9. *Aceria lepidosparti* Keifer, after Keifer. 10. *Aceria medicaginis* (Keifer), after Keifer. 11. *Aceria mori* (Keifer), after Keifer. 12. *Aceria nimia,* new species. 13. *Aceria slykhuisi* Hall. 14. *Aceria tulipae* (Keifer), after Keifer. 15. *Cecidophyopsis hendersoni* (Keifer), after Keifer. 16. *Eriophyes laevis* (Nalepa), after Keifer. 17. *Oxypleurites acidotus* Keifer, after Keifer. 18. *Phyllocoptes microspinatus,* new species. 19. *Platyphytoptus sabinianae* Keifer, after Keifer. 20. *Mesalox tuttlei* Keifer. 21. *Vasates cercidis,* new species. 22. *Vasates dimidiatus,* new species. 23. *Vasates laevigatae* (Hassan), after Keifer. 24. *Vasates lycopersici* (Massee), after Keifer. 25. *Vasates mckenziei* Keifer, after Keifer. 26. *Vasates micheneri,* new species. 27. *Vasates quadripedes* Shimer. 28. *Vasates quadripedes* Shimer. 29. *Phytoptus rotundus,* new species. 30. *Apodiptacus cordiformis* Keifer. 31. *Rhyncaphytoptus boczeki,* new species. 32. *Rhyncaphytoptus platani* Keifer, after Keifer.

ERIOPHYIDAE

Genus **Abacarus** Keifer

Abacarus Keifer, 1944, Bull. California Dept. Agr., 33:28.

Type of genus: Calepitrimerus acalyptus Keifer, 1939, Bull. California Dept. Agr., 28:490 (by original designation).

Discussion: In this genus the tergites form three dorsal, longitudinal, wax-bearing ridges. The central ridge is shorter than the laterals and terminates in a slight, dorsal depression. The setae, legs, and rostrum are not distinctly different from other genera. The genus *Calepitrimerus*, from which the type was segregated is similar to *Abacarus* but has the setiferous shield tubercles ahead of the rear margin of the shield. In *Abacarus* these tubercles are on the rear margin of the shield, with setae directed caudad.

This genus has three species; two from California and one from Austria. At present the genus is of no economic importance.

Abacarus sporoboli (Keifer)
(Plate 2)

Abacarus sporoboli Keifer, 1965. Eriophyid studies B-16. Bureau of Entomology, California Dept. Agr., p. 11.
Type locality: Some point in Sanborn Co., South Dakota.
Type host: Sporobolus airoides (Torr.) (Gramineae-Agrostidae) dropseed.

Relation to host: Keifer indicates the mites are probably leaf vagrants. In Kansas this species occurs on *Sorgum halepense* (L.) Pers. and there is no apparent damage to the host. On *S. halepense* mites are found scattered along the length of the leaf in the longitudinal furrows.

Discussion: Two species are rather similar to *Abacarus sporoboli* K. Differing in the shape of the female genital cover flap, featherclaw and papillose shield is *hystrix*. Another species, *afer*, from African coffee may be distinguished by noting that *sporoboli* has coarser granules on the shield, lacks side branches on the admedian lines, and 6-rayed featherclaw.

Kansas record: Stafford Co., Kansas, 30 mi. W. of Hutchinson, U.S. hwy. 50, south, Aug. 14, 1955, C. C. Hall. Collected from *Sorgum halepense* L. (Gramineae) Johnson grass.

Genus Aceria Keifer

Aceria Keifer, 1944, Bull. California Dept. Agr., 33:22.
Type of genus: Eriophyes tulipae Keifer, 1938, Bull. California Dept. Agr., 27:185 (by original designation).

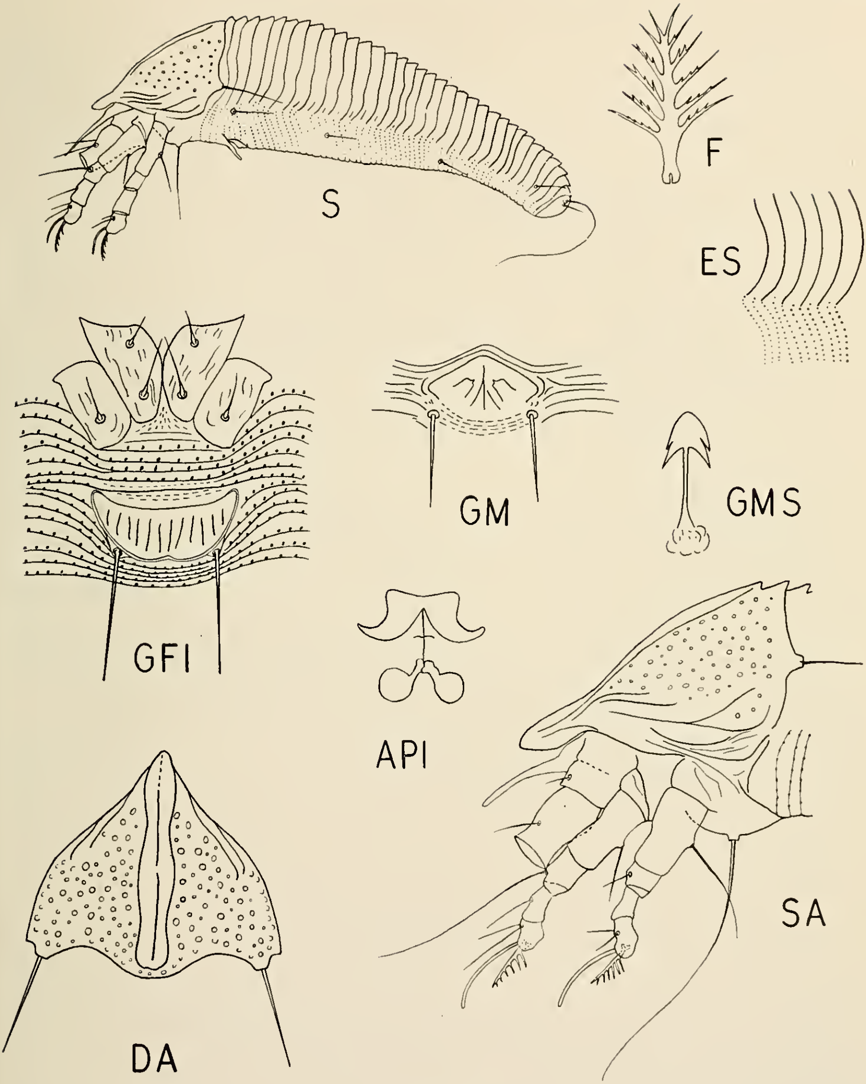
Discussion: This rather large genus includes wormlike mites with the dorsal setae situated on the rear margin of the shield and directed posteriorly. The rostrum is somewhat variable in size, but it is usually small and the chelicerae are always evenly curved. The tergites and sternites are similar with occasionally fewer tergites than sternites in the last third of the abdomen. The dorsal tubercles are frequently located with their long axes transverse to the body; this tends to cause the dorsal setae to diverge posteriorly. The axis of the featherclaw is undivided but variable in structure and number of rays.

This is a cosmopolitan genus which infests both mono- and dicotyledonous plants. All types of eriophyid injuries are produced by members of this genus, including the transmission of a plant virus disease by *Aceria tulipae.*

KEY TO THE SPECIES OF Aceria IN KANSAS

Aceria in Kansas contains more species than any other genus presently known in the area. Frequently a host association plus the type of damage is all that is needed to identify a species coming from a locality, especially if that area has been collected and certain species are known to occur there. For quick identification of Kansas *Aceria* a key is given below:

1. Featherclaws 3- to 4-rayed 2
1. Featherclaws 5- to 7-rayed 4



(Plate 2)

2. Featherclaws 3-rayed; shield with incomplete median and admedian lines (Microtubercles weakly expressed) .... *caryae*

2. Featherclaw with 3 or more rays; shield pattern lacking 3

3. Featherclaws 3-rayed; no markings on genital coverflap (Microtubercles distinct, rounded, and located in center of annular rings) .... *erineus*

3\. Featherclaws 4-rayed; 10 or 11 longitudinal marks on genital overflap .... *nimia*

4\. Featherclaws 5-rayed 5

4\. Featherclaws 6- or 7-rayed 10

5\. Genital flap with markings in two ranks (Shield with median, admedian, and submedian lines present; microtubercles very small, placed near posterior margin of annular rings; on (Opuntia) .... *cactorum*

5\. Genital flap without markings or with markings in one rank 6

6\. Genital flap without markings (Shield rounded and without markings; on *Celtis*) .... *celtis*

6\. Genital flap with 6 or more longitudinal markings in a single rank 7

7\. Genital flap with 6 or 7 longitudinal markings; shield with complete median and admedian lines, incomplete submedian lines; microtubercles oval and centrally located in annular rings (on *Ulmus*) .... *parulmi*

7\. Genital flap with more than 7 longitudinal markings; shield smooth or with a pattern; microtubercles present or absent 8

8\. About 13 longitudinal markings present on coverflap; shield smooth (Microtubercles posteriorly placed in annular rings, rounded, spinules present; on alfalfa) .... *medicaginis*

8\. About 10 longitudinal markings present on coverflap; shield pattern present 9

9\. Shield with median line indistinct, area between complete admedian lines filled with many broken lines, submedian lines irregular; microtubercles weakly expressed and in posterior region of annular rings .... *lepidosparti*

9\. Shield with median line distinct but broken, admedian lines complete, submedian lines weak and irregular; microtubercles oval .... *mori*

10\. Featherclaws 6-rayed (About 8 or fewer markings on genital flap; microtubercles rounded and in center of annular rings) .... slykhuisi

10\. Featherclaws 7-rayed 11

11\. Shield without markings, narrowed anteriorly; microtubercles small, located on posterior margin of annular rings (on bermuda grass) .... *cynodonis*

11\. Shield with median line incomplete, admedians complete, and submedians irregular; microtubercles of average size, located in posterior half of annular rings .... *tulipae*

Aceria parulmi (Keifer)
(Plate 3)

Aceria parulmi Keifer, 1965. Eriophyid studies B-13. Bureau of Entomology, California Dept. Agr., p. 9.
Type locality: Beloit, Wisconsin.
Type host: Ulmus americana L.

Relation to host: Fingerlike galls are produced on the upper leaf surfaces. This is also true for specimens collected in Kansas from the same host species. The Kansas material had galls of various sizes and mites were very numerous. Even when galls were abundant the host plant was not seriously injured. Galls on young leaves had the same green color as the leaves but on older leaves, galls were frequently brownish or dark in color. The number of galls varied from two to 25 or 30 per leaf. The infestation seemed localized on the host with only a few leaves in any single area showing galls.

Kansas record: Lawrence, Douglas Co., Kansas, Oct. 28, 1954, C. C. Hall (on the University of Kansas campus).

Aceria cactorum (Keifer)
(Plate 4)

Eriophyes cactorum Keifer, 1938, Bul. California Dept. Agr., 27:185.
Aceria cactorum (Keifer), Keifer, 1952, Bull. California Insect Survey, 2:25.
Type locality: Santa Paula, Ventura Co., California.
Type host: Opuntia sp.

Relation to host: No noticeable damage is reported to the host even though mites may be abundant on developing flowers and new pads. In Kansas this species is not abundant and specimens were difficult to find. The only records for this species are from Kansas and California.

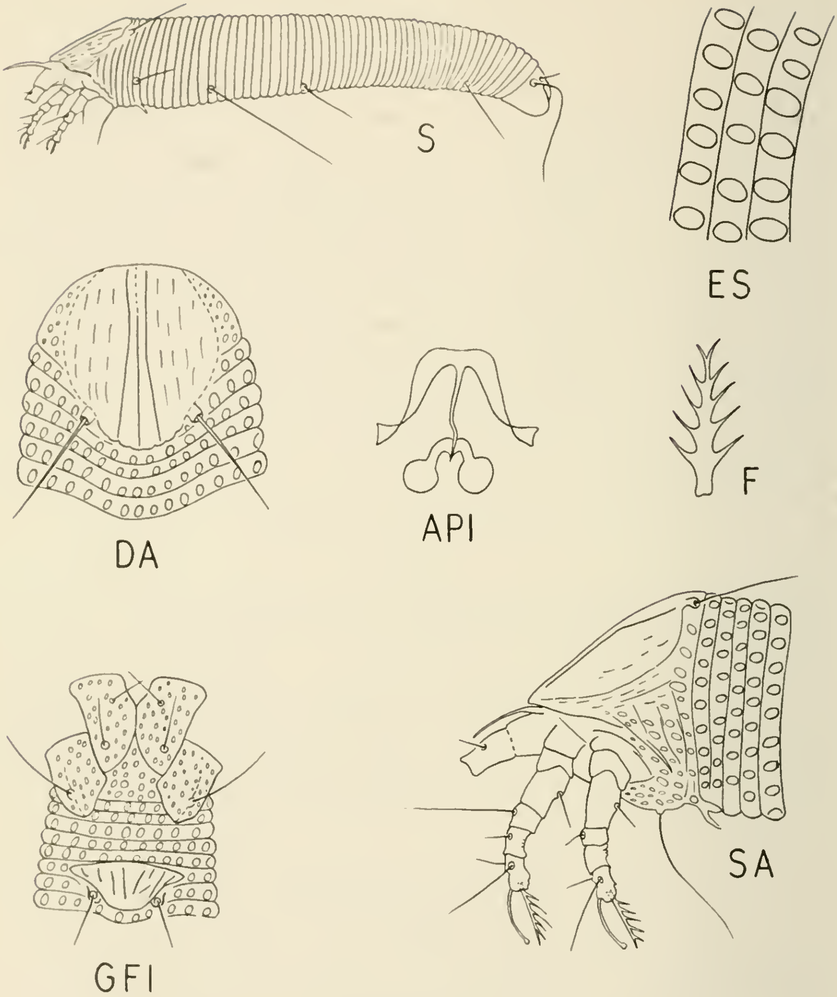
Kansas record: Lawrence, Douglas Co., Aug. 5, 1954, C. C. Hall (from *Opuntia* pads).

Aceria caryae (Keifer)
(Plate 5)

Eriophyes caryae Keifer, 1939, Bull. California Dept. Agr., 28:484.
Type locality: Brownwood, Texas.
Type host: Carya illinoensis (Wang), K. Koch, pecan.

Relation to host: A marginal leaf-roll on the upper surface is the type of damage produced by the species in all collected areas. These marginal deformities may be numerous or only a few present on a tree. The mites live in a large mass of spongy tissue produced inside the roll. Serious damage to pecan trees by this species has not been reported. This kind of injury was reported by Keifer (1939) for various *Carya* species.

Discussion: Aceria caryae and *Aceria erineus* (Nalepa) are very similar. The different hosts, walnut or hickory for *A. erineus* and pecan for *A. caryae*, indicate that these are perhaps good species. Morphological differences are chiefly seen in the shield, dorsal tubercles, and genital apodemes of the



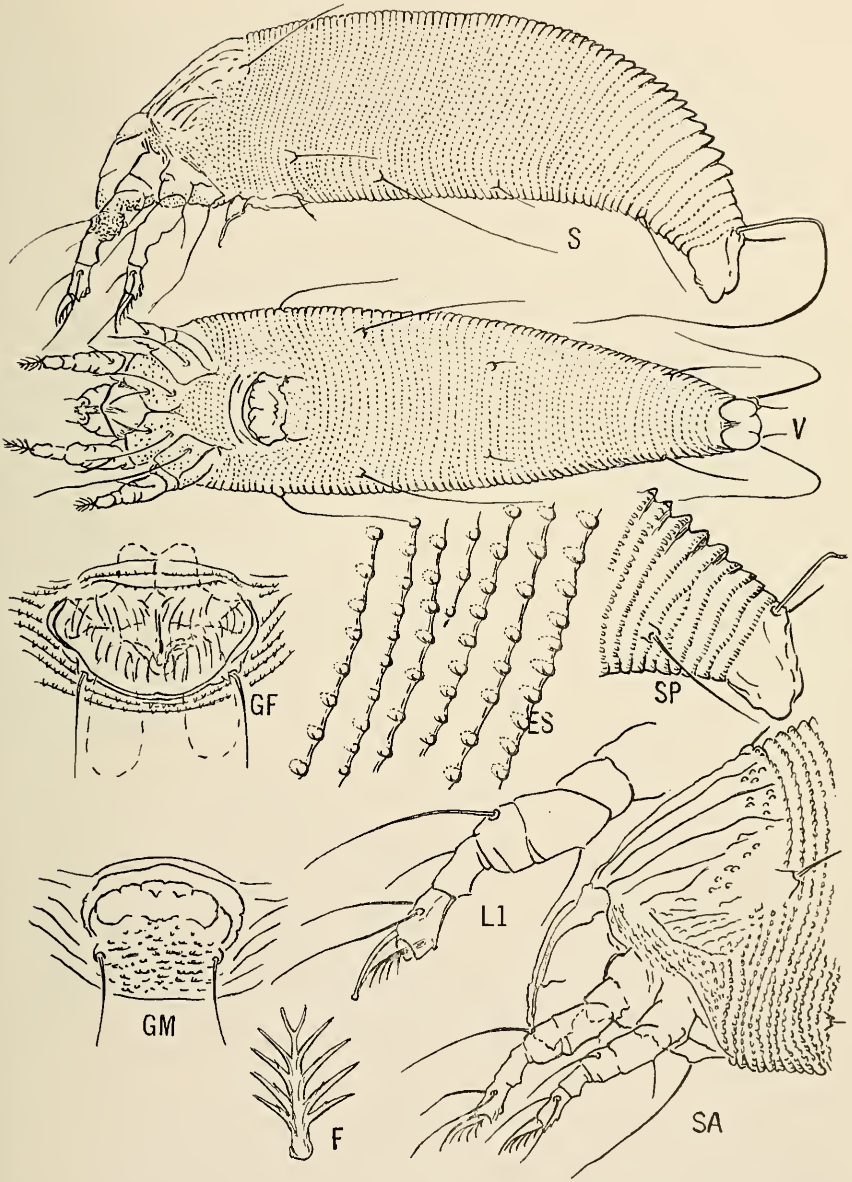
(Plate 3)

female. *A. caryae* also has body rings that are nearly smooth while the closely related species has microtubercles more distinct.

Kansas record: Baxter Springs, Cherokee Co., Oct. 9, 1954, C. C. Hall (from pecan).

Aceria celtis (Kendall)
(Plate 6)

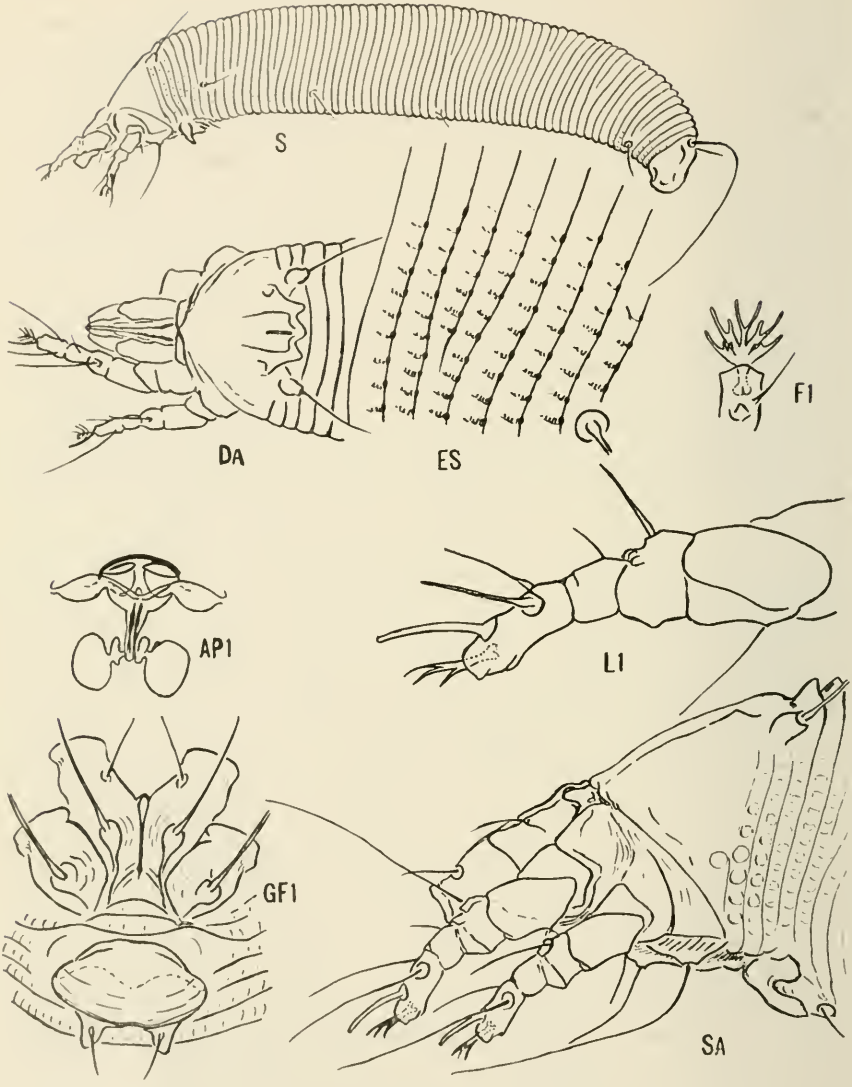
Eriophyes celtis Kendall, 1929, Psyche, 36:300.
Type locality: Forest Hills, Massachusetts.
Type host: Celtis occidentalis L., *Celtis occidentalis canina.*



(Plate 4)

Aceria snetsingeri Keifer, 1957, Bull. California Dept. Agr., 46:244 (new synonymy). *Type locality:* Bradley, Illinois. *Type host: Celtis occidentalis* L., hackberry.

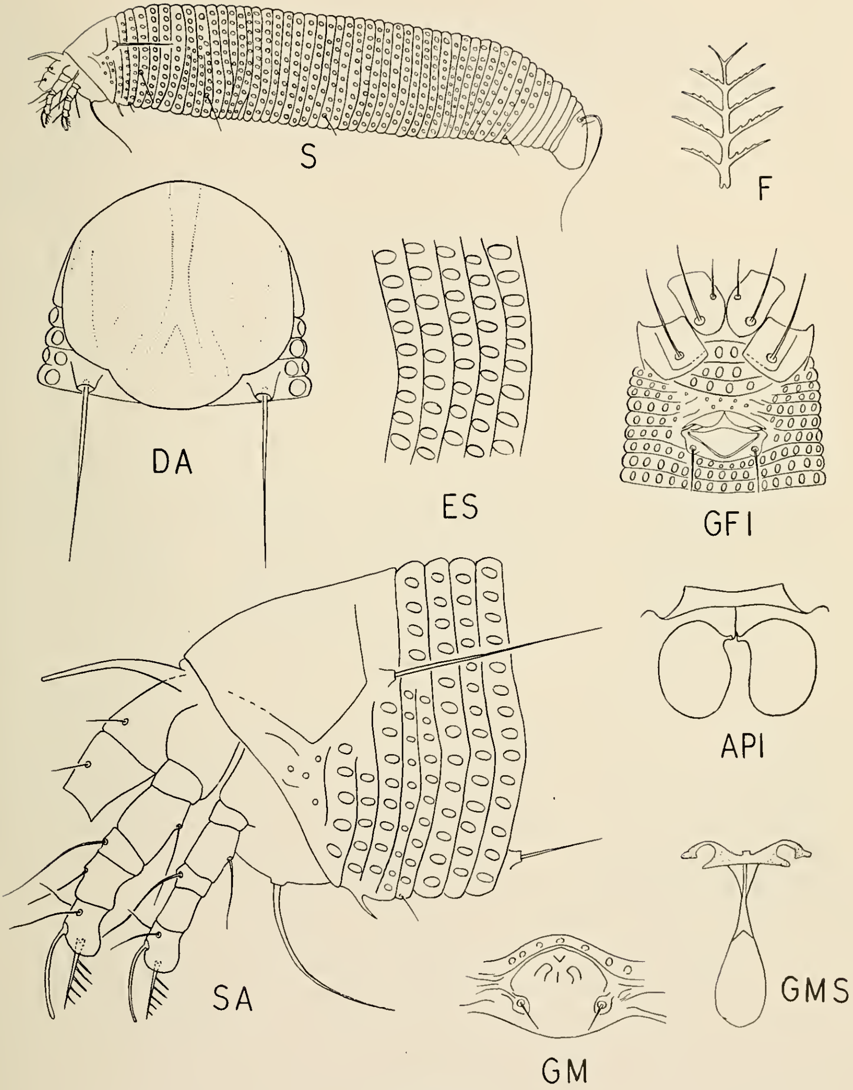
Relation to host: Bud deformation and "witches'-broom" development are the symptons shown by the host when infested with this species. Kendall



(Plate 5)

(1929) and Keifer (1957) report the type of damage mentioned above on the host plant. In Kansas the injury to the host is the same, and the photograph by Keifer (1957) could not be distinguished from a Kansas specimen. Mites may be taken from these "witches'-brooms" any time of the year.

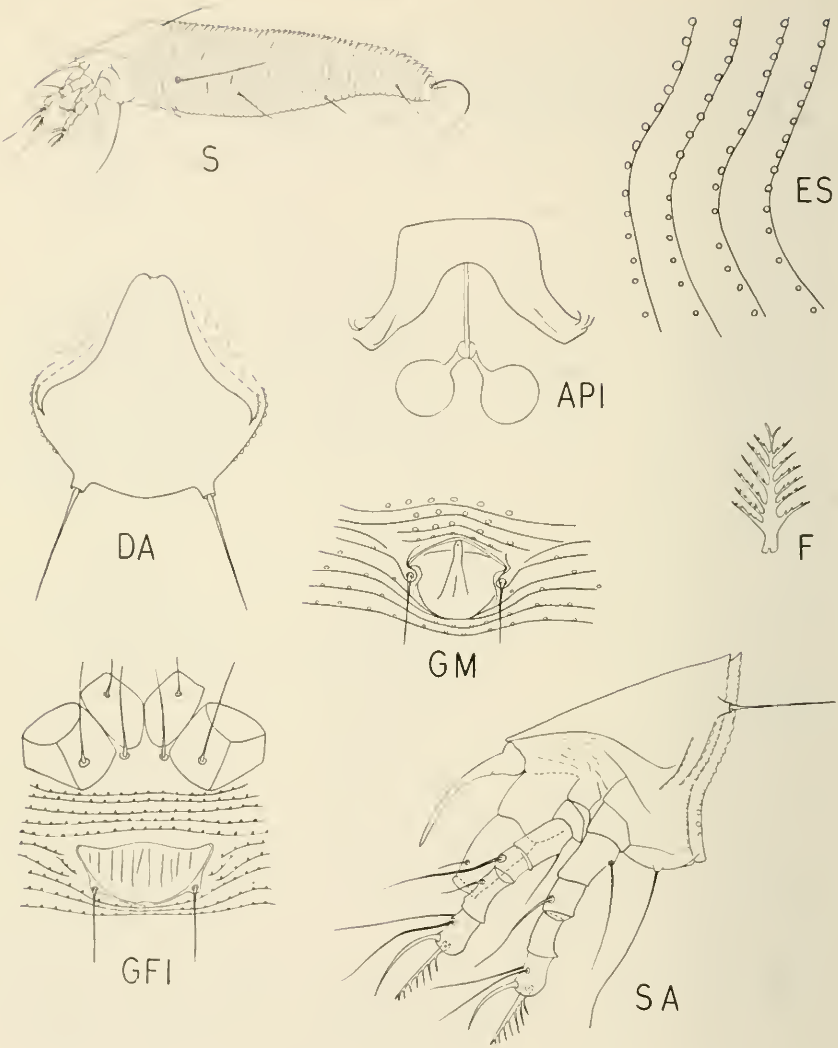
Discussion: This is apparently a widespread species following the range of its host. The writer has observed the typical damage throughout Kansas,



(Plate 6)

and it seems common also in Oklahoma and Texas. Specimens should be examined from the type locality and from several other areas to be sure this is a single species.

Kansas records: Lawrence, Douglas Co., Aug. 5, 1954, C. C. Hall (from hackberry "witches'-broom"); several samples of "witches'-brooms" from Riley Co. contained specimens of *Aceria celtis.*

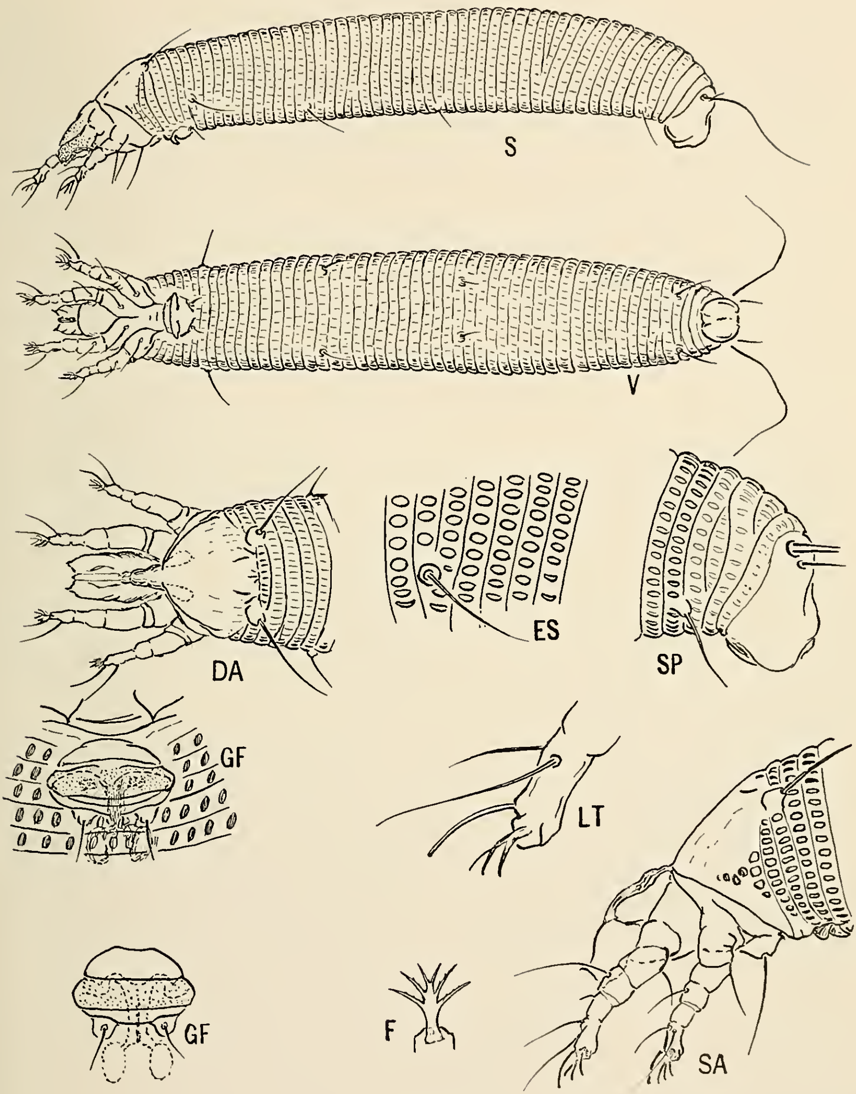


(Plate 7)

Aceria cynodonis Wilson
(Plate 7)

Aceria cynodonis Wilson, 1959, Ann. Ent. Soc. America, 52:142.
Type locality: Moreno, Riverside Co., California.
Type host: *Cynodon dactylon* (L.) Pers., bermuda grass.

Relation to host: Wilson (1959) describes the damage as mostly twisting of the folded terminal shoot with subsequent infolding and twisting of the expanded blade. Infested grass is easily recognized if it is allowed to grow



(Plate 8)

freely without cutting. The terminal loops formed by the weakened, distorted shoots are heavily infested with mites. This is similar to the condition exhibited by wheat that is infested with *Aceria tulipae.*

Discussion: There is no difficulty in separating this species from other *Aceria.* The shield design is characteristic and with the host reference *Aceria cynodonis* is quickly identified. Kansas material, kept in the greenhouse, developed a heavy infestation which caused some stunting of the grass. Some shoots formed five or six successive loops. All stages of development were present on the grass. Eggs were ovoid, transparent, and deposited in abundance in furrows on the inner surfaces of the leaves. Immatures were transparent to opaque. Larger, mature mites were darker in color but became no darker than a light brown. These mites were observed climbing plant hairs and extending their bodies into space with the posterior end of the mite attached to the plant hair; this was also done on the leaf surface. See behavior section for further discussion of this activity.

Kansas record: Manhattan, Riley Co., April 8, 1958, Salome del Rosario (from Bermuda grass).

Aceria erineus (Nalepa)
(Plate 8)

Phytoptus tristriatus erineus Nalepa, 1891, Anz. Akad. Wiss. math-nat. Wien, 28:162.
Aceria erineus (Nalepa), Keifer, 1952, Bull. California Insect Survey, 2:27.
Type locality: Austria?
Type host: *Juglans regia* L., Persian or English walnut.
Additional host: *Carya* sp., hickory, Franklin Co. Kansas.

Relation to host: The Kansas host material responds to the presence of this mite by producing a marginal leaf roll that is internally a mass of hairs in which the mites live. Damage to the host has been noted only as slight. On the type host, *Juglans regia* L., large masses of thick hair are produced on the lower surface of the leaf (Keifer, 1952).

Discussion: The most unique character of this species is the presence of genital tubercles. The shape of the genital coverflap is unusual. Actually this species seems close to *Aceria caryae* (Keifer) but has distinct microtubercles, a slightly different featherclaw, and no shield pattern. This is apparently another widely distributed species as indicated by its occurrence in Austria, California, and Kansas. Records are common in California and specimens are easily found on hickory in Kansas.

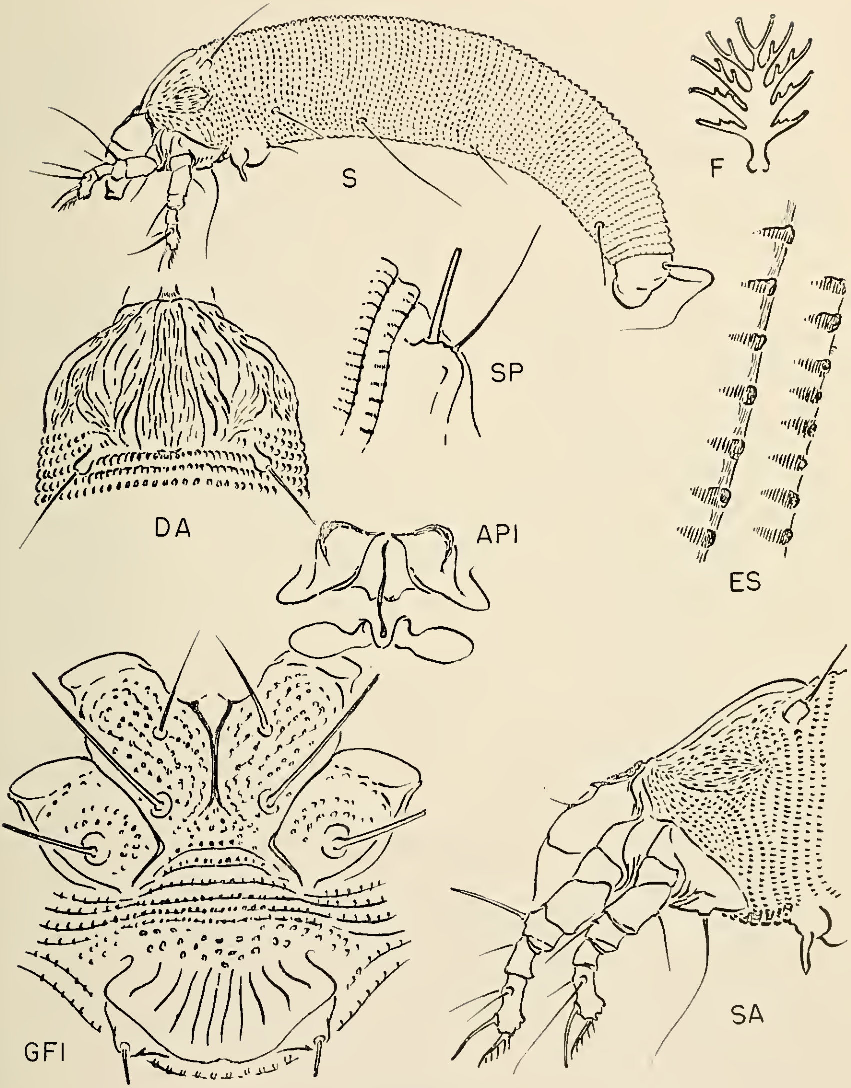
Kansas record: Franklin Co., May 14, 1954, C. D. Michener (from hickory).

Aceria lepidosparti Keifer
(Plate 9)

Aceria lepidosparti Keifer, 1951, Bull. California Dept. Agr., 40:95.
Type locality: San Bernardino (Devore district), San Bernardino Co., California.
Type host: *Lepidospartum squamatum* Gray.

Relation to host: On the type host, bud clusters are produced on the stems and stunting of growth occurs at that point. The Kansas host, *Morus rubra* L., did not show any abnormal growth except minor distortion of leaves.

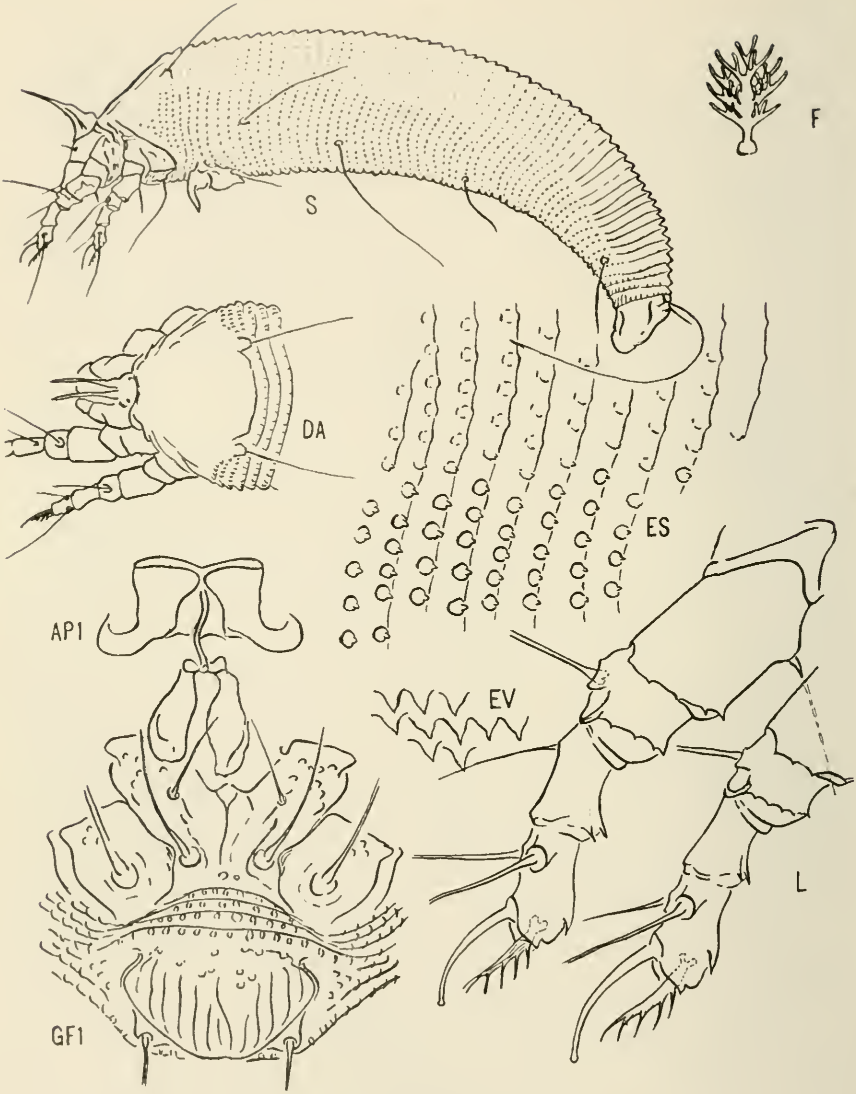
Discussion: A few specimens were obtained from Kansas material and, no morphological differences could be found between Kansas and California material even though hosts are greatly separated. Paucity of specimens and



(Plate 9)

excessive clearing in Hoyer's mounting medium have made the determination difficult. Specimens were examined carefully before excessive clearing took place and determined as *Aceria lepidosparti* or very near this species. The species has been collected only in Kansas and California.

Kansas records: Stafford Co., Aug. 14, 1955, C. C. Hall (on *Morus rubra* L., mulberry).

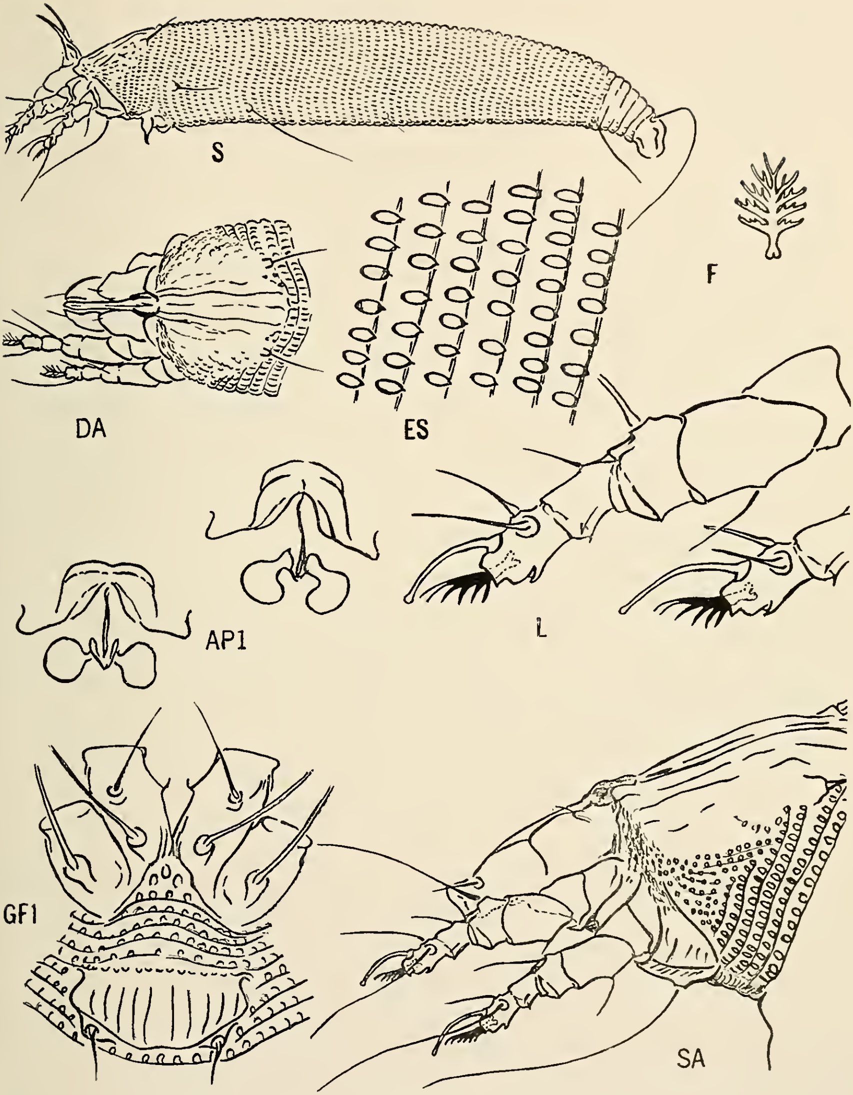


(Plate 10)

Aceria medicaginis (Keifer)
(Plate 10)

Eriophyes medicaginis Keifer, 1941, Bull. California Dept. Agr., 30:206.
Aceria medicaginis (Keifer), Keifer, 1952, Bull. California Insect Survey, 2:30.
Type locality: Sacramento, Sacramento Co., California.
Type host: Medicago sativa Linnaeus, alfalfa.

Relation to host: No damage has been reported from this species. Some-



(Plate 11)

times specimens are numerous but only slight growth deformity seems to result from this species. It is possible that it causes occasional flower damage but apparently not enough to consider it economically important. The mites live in the leaf axils and the buds.

Discussion: This is a typical *Aceria.* There is some suggestion of increase in the width of the tergites so that they are just slightly wider than the sternites. The lack of a shield pattern is also useful in identifying this species. *A. medicaginis* has been collected only in California and Kansas.

Kansas records: Marshall Co., Dec. 25, 1955, D. L. Matthew (from alfalfa): Hays, Ellis Co., Jan. 11, 1956, T. L. Harvey (from alfalfa).

Aceria mori (Keifer)
(Plate 11)

Eriophyes mori Keifer, 1939, Bull. California Dept. Agr., 28:485.
Aceria mori (Keifer), Keifer, 1952, Bull. California Insect Survey, 2:31.
Type locality: Sacramento, Sacramento Co., California.
Type host: *Morus* sp.
Additional host: *Morus* sp., Baxter Springs, Cherokee Co., Kansas. See relation to host and discussion.

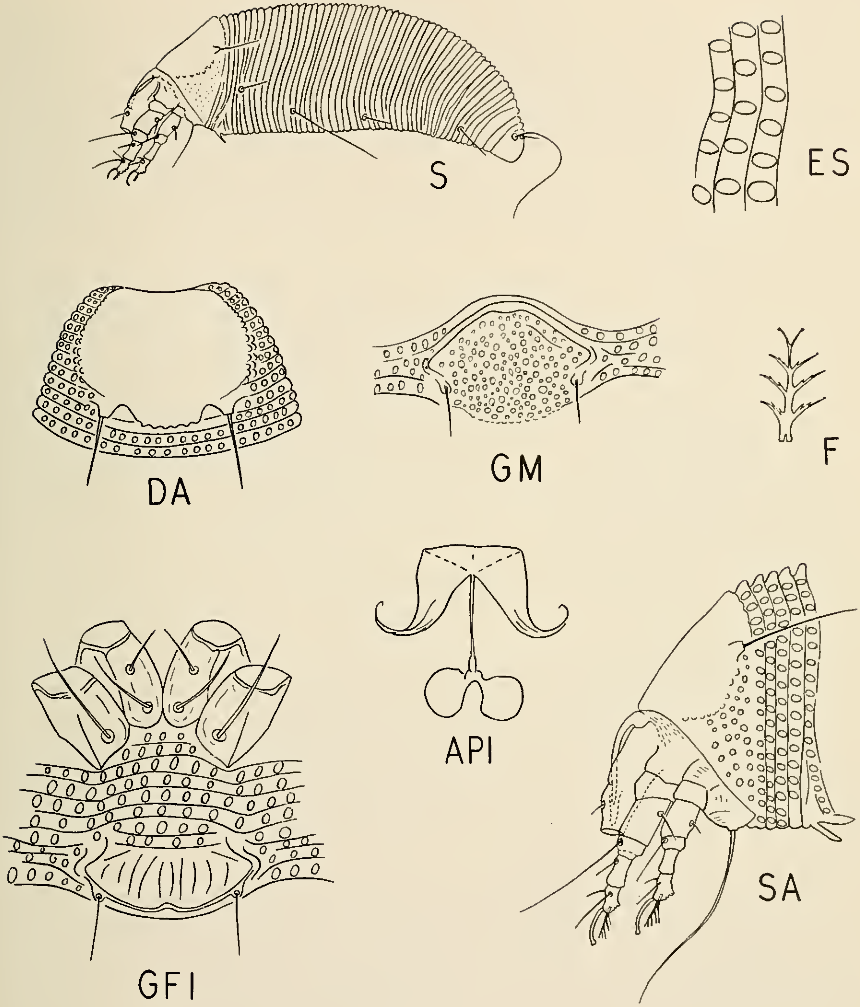
Relation to host: The California host shows some fruit deformity, but it is not certain that eriophyids are the cause. In Kansas the tree from which specimens were collected appeared to have lost all of its leaves, and new, distorted tufts of leaves were showing up, usually near the trunk. These tufts were infested with eriophyids, and occasionally mites could be found on the lower surface of some leaves.

Discussion: Similarities are striking between this species and other *Aceria*. *A. feijoae* (Keifer), *A. lepidosparti* Keifer, and *A. diospyri* Keifer can easily be confused with *A. mori*. The host, shield pattern, genital coverflap and microtubercles must be examined carefully to recognize this species. This species is known only from California and Kansas.

Kansas record: Baxter Springs, Cherokee Co., Oct. 9, 1954, C. C. Hall (from *Morus* sp.).

Aceria nimia, new species
(Plate 12)

Female: 167-170 μ long, 20 μ wide, wormlike, slightly arched in lateral view. Rostrum 22 μ long, straight, directed downward at slight angle to body. Chelicerae 15 μ long, curved evenly. Shield 21 μ long, 18 μ wide, without markings, rounded, posterior and lateral margins crenate. Dorsal setae 17 μ long, 18 μ apart. Dorsal tubercles projected from rear margin of shield. Forelegs 20 μ long; femur 7 μ long, seta 8 μ long; genu 5 μ long, seta 20 μ long; tibia 7 μ long, seta 6 μ long; tarsus 5 μ long, outside seta 18 μ long. Claw 8 μ long, curved, small knob at tip. Axis of featherclaw undivided, 4-rayed, shorter than claw. Hind legs 20 μ long; femur 7 μ long, seta 7 μ long; genu 4 μ long, seta 6 μ long; tibia 4 μ long, without a seta; tarsus 4 μ long, outside seta 18 μ long. Claw 8 μ long, curved, small knob at tip. Axis of featherclaw undivided, 4-rayed. Anterior coxae contiguous at posterior third; posterior coxae strongly emarginate, covering part of anterior coxae. Abdomen with 80 tergites and sternites. Microtubercles ovoid, papillose, centered in annular ring, similar in all areas, varying only in size. Female



(Plate 12)

genitalia 18 μ wide, 10 μ long, 9 or 10 longitudinal scorelines, setae 5 μ long. Apodemes strongly produced, spermathecae rounded.

Male: 130 μ long, similar to female. Genital coverflap papillose with slight indentation anteriorly.

Type locality: Lawrence, Douglas Co., Kansas, July 19, 1958, C. C. Hall, The University of Kansas campus in front of the library.

Type host: Fraxinus americana L. (Oleaceae).

Relation to host: Damage to the host is confined to the fruiting bodies which become rough, irregular, pendant masses of tissue. Mites are present in these distorted fruiting bodies.

Location of types: Female holotype and three paratype slides from the type locality bearing above data are deposited in Snow Entomological Museum, The University of Kansas, Lawrence. A paratype slide bearing the same data has been sent to H. H. Keifer, California Department of Agriculture, Sacramento, Calif. One paratype slide with the same data has been sent to the U.S. National Museum. Seven paratype slides collected May 21, 1954, C. C. Hall, from the same tree as well as dry paratype material are in the author's collection.

Discussion: The lack of figures and specimens for comparison make it difficult to give a statement regarding related species. Material sent to H. H. Keifer was considered by him to be new. Information gleaned from keys indicates that *Aceria fraxinivorous* (Nalepa) has a distinct shield pattern and the species described here has a shield without markings. The name of this species is formed from the Latin word *nimius* which means excessive and refers to the masses of distorted fruiting bodies produced by the host in response to the presence of this mite.

Aceria slykhuisi Hall
(Plate 13)

Aceria slykhuisi Hall, 1958, Jour. Kansas Ent. Soc., 31:233.
Type locality: Hays, Ellis Co., Kansas.
Type host: Buchloe dactyloides (Nutt.) Engelm., buffalo grass.

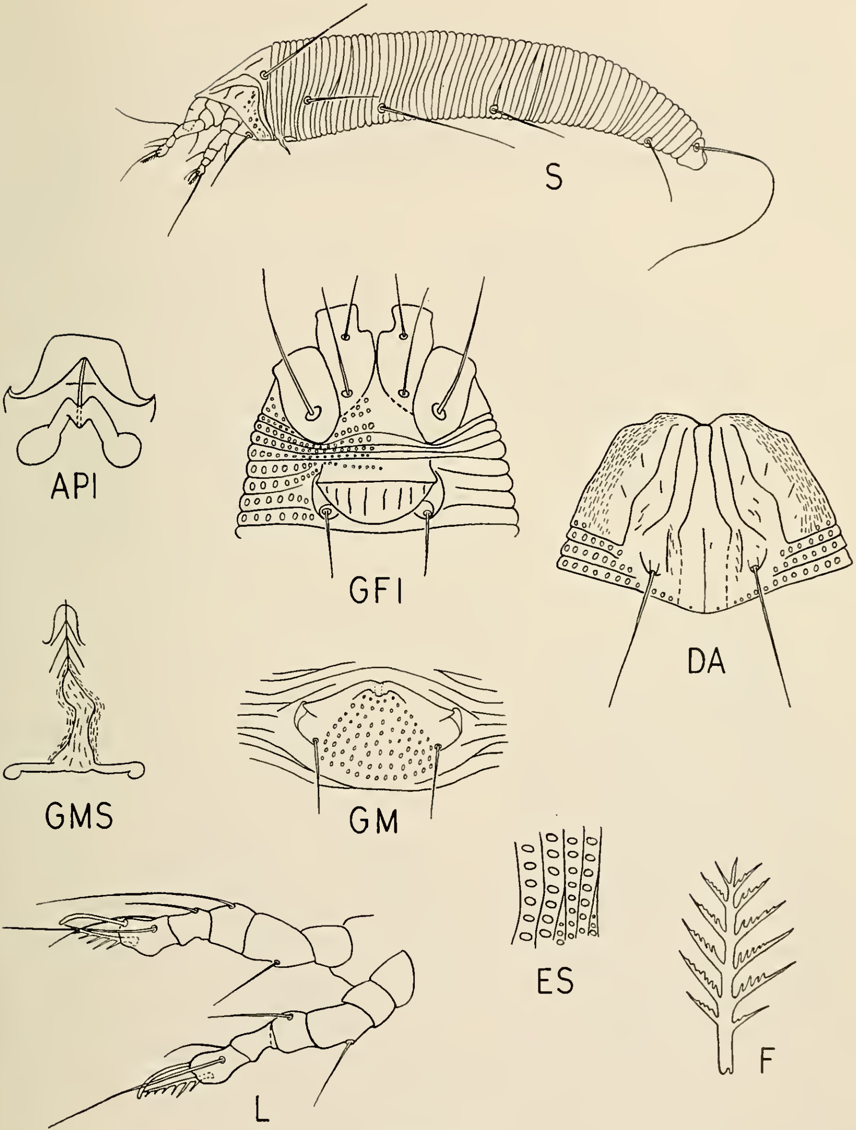
Relation to host: Witchbrooming seems to be caused by the presence of this mite on the pistillate plants. The presence of tarsonemid mites in these deformities is probably secondary since they appear later than the eriophyids. The relationship between tarsonemids and eriophyids is discussed by Hall (1958).

Discussion: This species is similar to *Aceria tulipae* but can be distinguished by the microtubercles, featherclaw, and genital coverflap. *A. slykhuisi* has been collected only from the type host, buffalo grass, and *A. tulipae* has been collected from several hosts. The host plant *Buchloe dactyloides* (Nutt.) Engelm. occurs throughout western Kansas where it is a dominant grass.

Kansas record: Hays, Ellis Co., Aug. 12, 1954, T. L. Harvey (from buffalo grass at Kansas Agr. Exp. Station).

Aceria tulipae (Keifer)
(Plate 14)

Eriophyes tulipae Keifer, 1938, Bull. California Dept. Agr., 27:185.
Aceria tulipae (Keifer), Keifer, 1952, Bull. California Insect Survey, 2:33.
Type locality: Sacramento, Sacramento Co., California.
Type host: Tulipa sp.
Additional hosts: Allium cepa L., *Allium sativa* L., *Agropyron smithii* Rydb., *Hordium pubatum* L., *Muhlenbergia racemosa* (Mich.) B.S.P., *Bromus maritimus* (Piper) Hitchc., *Bromus tectorum* L., *Digitaria sanguinalis* L., *Elymus canadensis* L., *Hordeum leporinum* Link., *Lolium*

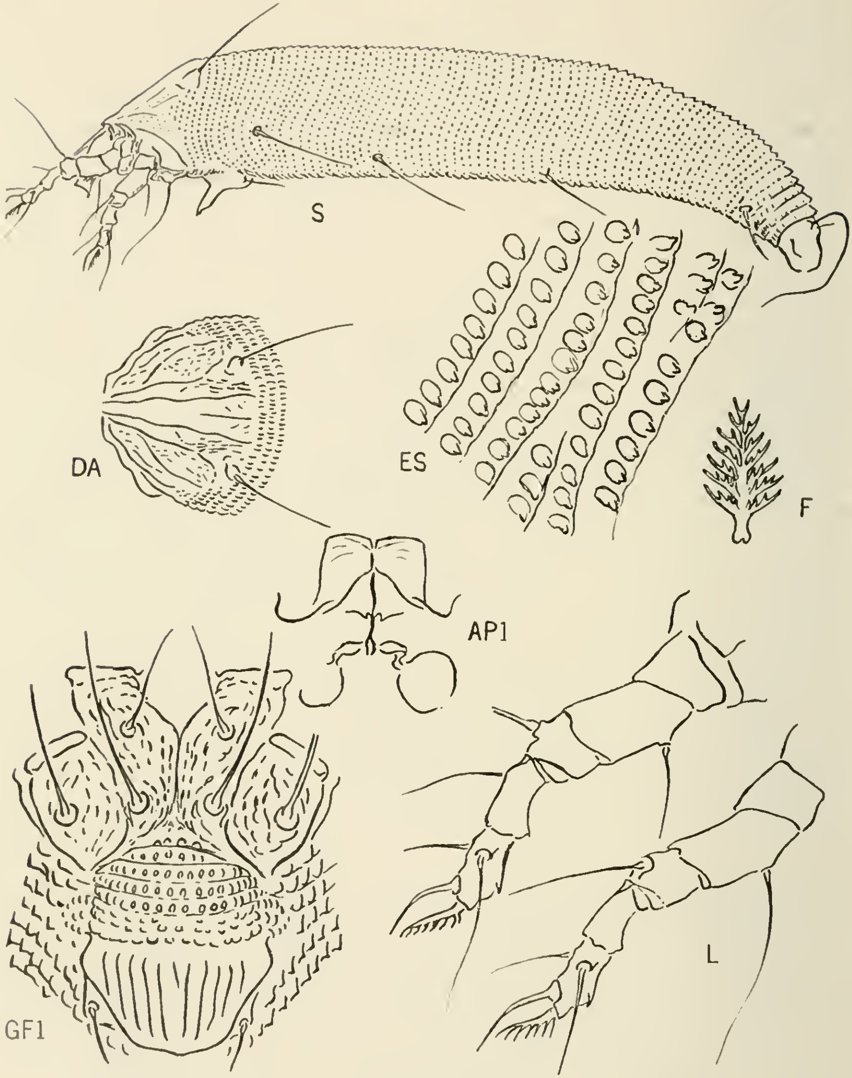


(Plate 13)

sp., *Setaria glauca* (L.) Beauv., *Setaria viridis* (L.) Beauv., and several varieties of wheat. Kansas records include onion, barley, wheat, and western wheatgrass.

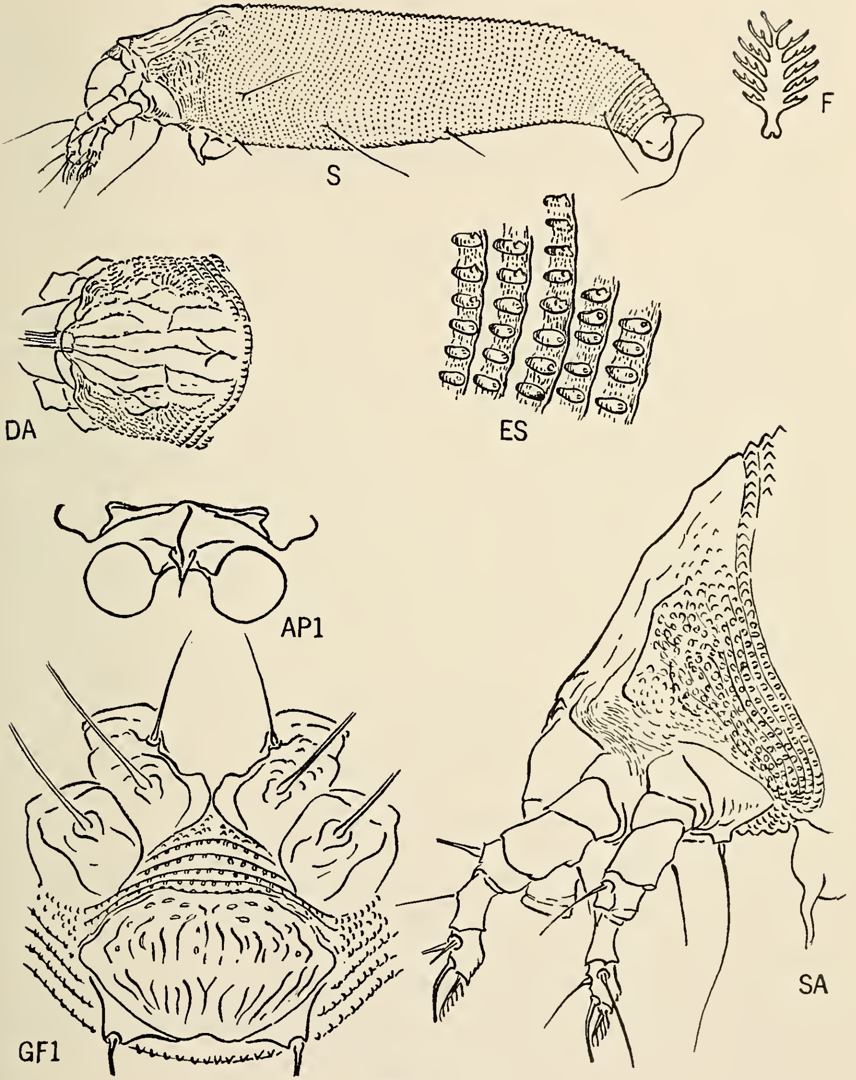
Relation to host: On most hosts of this species the "caught leaf" condition indicates the presence of mites.

Discussion: This species is the most important, economically, of all eriophyids since it serves as the vector of the wheat streak mosaic virus. *Aceria*



(Plate 14)

tulipae occurs throughout Kansas and is found in both North America and Europe. Keifer (1952) indicated common occurrence of this species on garlic imported from Mexico. Experiment stations are especially concerned with this economically important species, and useful information on biology and behavior can be gleaned from these reports and papers. *Aceria tulipae* still has not been thoroughly studied on all host plants and all host relationships are not clear.



(Plate 15)

Kansas records: Manhattan, Riley Co., March 7, 1954, R. E. Beer (wheat in greenhouse); Ottawa, Franklin Co., April 20, 1954, C. C. Hall (on volunteer wheat); Hays, Ellis Co., April 20, 1954, T. L. Harvey (from western wheatgrass); Lawrence, Douglas Co., May 11, 1954, C. C. Hall (wheat in the field); several field collections from Riley Co. and Ellis Co. Obviously this species occurs throughout the state, but it is more common in the western wheat fields.

Genus **Cecidophyopsis** Keifer

Cecidophyopsis Keifer, 1959, Bull. California Dept. Agr., 47:273.

Type of genus: Eriophyes vermiformis Nalepa, 1889 (by original designation).

Discussion: This is the only genus in the Eriophyidae that lacks the dorsal setae. The absence of shield setae, the genitalia close to coxae, the coxae slightly separated, and the coverflap with scorelines partly in two ranks are the more important characteristics of the genus.

This is a small but probably widespread genus with species in Oregon, California, Kansas, and several probable European species (Keifer 1959).

Cecidophyopsis hendersoni (Keifer)

(Plate 15)

"*Cecidophyes*" *hendersoni* Keifer, 1954, Bull. California Dept. Agr., 43:123.
Type locality: Syracuse, Hamilton Co., Kansas.
Type host: Yucca glauca Nutt., yucca.
Cecidophyopsis hendersoni Keifer, 1959, Bull. California Dept. Agr., 47:275.

Relation to host: A slight browning at the base of outer leaves where these mites live seems to be the only damage. This has been observed by Keifer (1954) and the writer. There is no extensive injury to the host.

Discussion: The possession of a 6-rayed featherclaw by *hendersoni* distinguishes it from *malpighianus* Nalepa, *psilaspis* Nalepa, *verilicis* Keifer, and *vermiformis* Nalepa which are all 5-rayed. Kansas and California are the only areas in which this species has been collected.

Kansas record: Syracuse, Hamilton Co., Aug. 14, 1955, C. C. Hall (from *Yucca glauca* Nutt.).

Genus **Eriophyes** von Siebold

Eriophyes von Siebold, 1950, Jahresber. Schles. Ges., 28:89.
Type of genus: Eriophyes vitis (Pagenstecher), (by subsequent designation of Keifer, 1938, Bull. California Dept. Agr., 27:301).

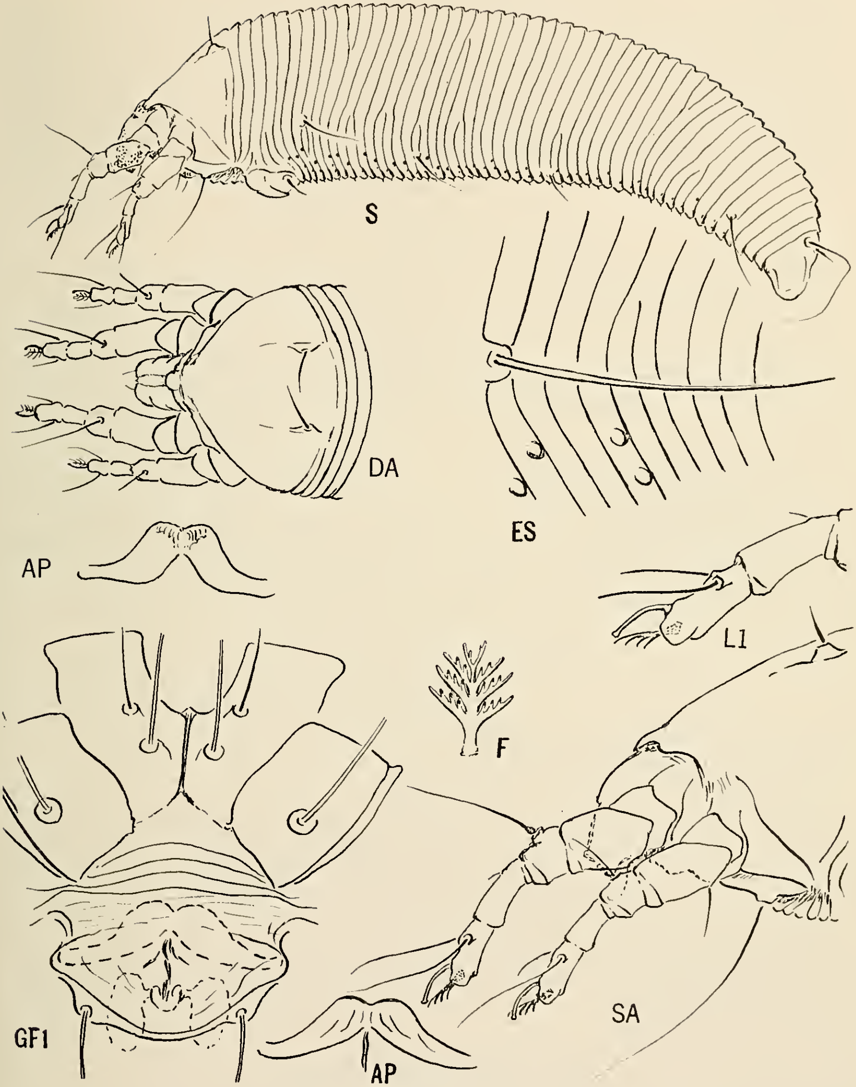
Discussion: The genera *Aceria* and *Eriophyes* are somewhat similar. In *Aceria* the dorsal setae are located on the rear margin of the shield and directed caudally. Members of *Eriophyes* have the dorsal setae situated on tubercles slightly ahead of the rear margin of the shield and directed upward and forward or centrally.

Mites of this genus cause a variety of growth deformities. The common occurrence of gall and blister mites in this genus marks it as an economically important group. It is world-wide in distribution.

Eriophyes laevis (Nalepa)

(Plate 16)

Phytoptus laevis Nalepa, 1889, Sb. Akad. Wiss. Math-nat. Wien, 98:132.
Type locality: Austria.
Type host: Alnus glutinosa L.
Eriophyes laevis (Nalepa), 1898, Das. Tierreich, 4th Issue: Acarina, p. 7, Berlin. *Type locality:* Middle Europe (presumed locality). *Type host: Alnus glutinosa* L.
Eriophyes rhombifoliae Hassan, 1928, Univ. California Publ. Ent., 4:378. *Type locality:* Yosemite Valley, California. *Type host: Alnus rhombifolia* Nutt.
Eriophyes marinalni Keifer, 1939, Bull. California Dept. Agr., 28:223. *Type locality:* Stinson Beach, Marin Co., California. *Type host: Alnus oregona* Nutt., Red alder.



(Plate 16)

Eriophyes laevis (Nalepa) Keifer, 1952, Bull. California Insect Survey, 2:37. *Type locality:* Austria. *Type host: Alnus glutinosa* L., alder.

Additional hosts: Alnus oregona Nutt. and *Alnus tenuifolia* Nutt., both of California. *Betula pubescens* Ehrhart, *Alnus glutinosa* L., *Alnus incana* DC., and *Alnus pubescens* Tausch in Europe.

Relation to host: On the type host this species causes beadlike galls to form on the leaves. Small bead galls were also noted on *Salix* sp., the Kansas host. Damage to the Kansas host was very slight and only a few galls were collected. *Eriophyes laevis* and *Eriophyes emarginatae* Keifer are two distinct species that may be separated by examining the genital apodemes of the females. In *laevis* the genital apodeme is broad and short; *emarginatae* has an apodeme that is narrow and long. Austria, California, and Kansas are the only areas in which this species has been collected.

Kansas record: Baxter Springs, Cherokee Co., Oct. 9, 1954, C. C. Hall (from *Salix* sp.).

Genus **Oxypleurites** Nalepa

Oxypleurites Nalepa, 1891, Denk. Akad. Wiss. math-nat. Wien, 58:868.

Type of genus: Oxypleurites trouessarti (Nalepa), 1923, Verhandlungen Zool.-bot. Gesselschaft, Wien, 72:15.

Discussion: The most distinctive characteristic of this genus is the presence of lateral toothlike projections of the tergites. The tergites are much broader and less numerous than the sternites. The dorsal setae vary in position.

There are about 15 described species in this genus; most of them are from California and one species, *O. simus* Keifer, from North Carolina. There are a few European species indicating that this is another genus of wide range. It seems not to be of great economic importance; rusting, browning, and silvering of leaves are reported for various species.

Oxypleurites acidotus Keifer
(Plate 17)

Oxypleurites acidotus Keifer, 1939, Bull. California Dept. Agr., 28:493.

Type locality: San Francisco, California.

Type host: Baccharis pilularis DC., chaparral broom.

Relation to host: This is a vagrant species, chieflly on the upper surface of older, less viscid leaves on the type host. On *Morus*, the Kansas host, mites were taken from the lower surface of the leaf along veins and scattered. There were no deformities of foliage noted.

Discussion: In such a distinct genus with only a few species, comparison of genitalia separates species readily. Dorsal setae and tergites are also useful in determining species.

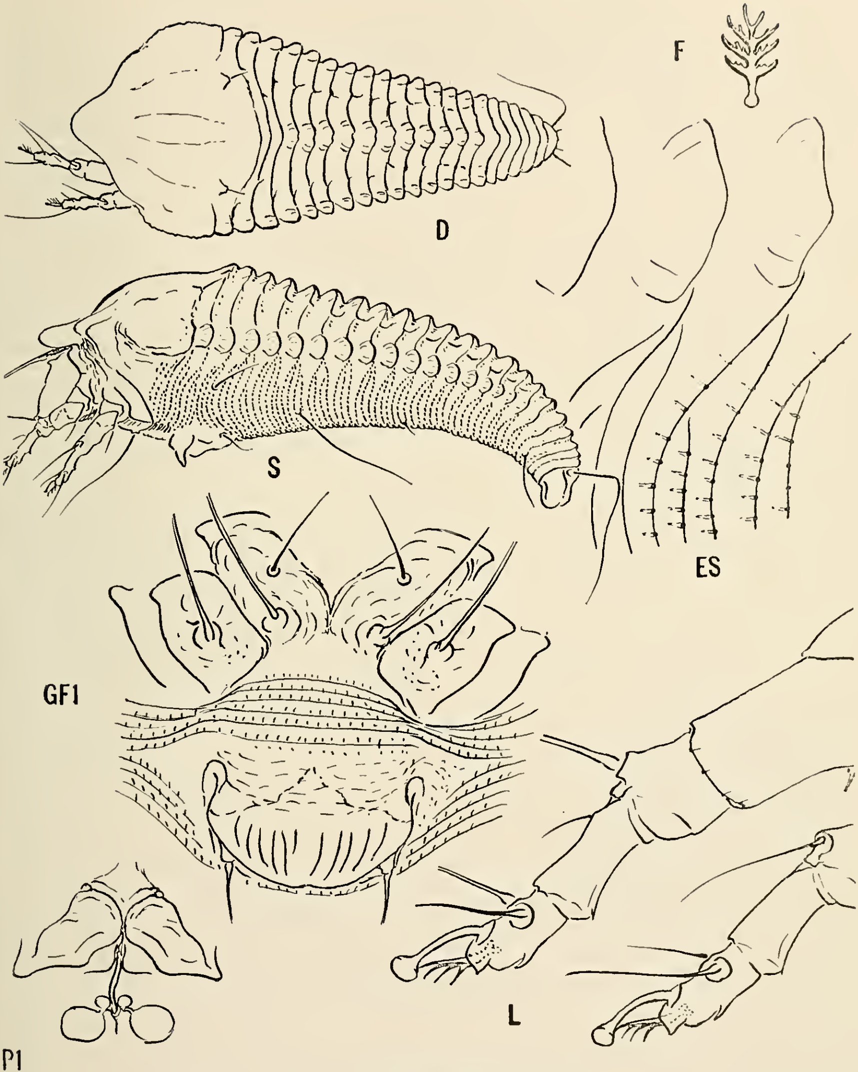
Genus **Phyllocoptes** Nalepa

Phyllocoptes Nalepa, 1889, Sitzb. Akad. Wiss. math-nat. Wien, 98:116.

Type of genus: Phyllocoptes carpini Nalepa (by subsequent designation of Keifer, 1938, Bull. California Dept. Agr., 27:191).

Discussion: This genus may be recognized by the undivided featherclaws; dorsal setae ahead of rear margin of shield, directed centrally, upward, or forward; and the presence of slight subdorsal furrows on the abdomen.

Many species in this genus are vagrant forms, living usually on the lower surfaces of leaves. Buds, petiole bases, and fruit are also reported as areas where the species may be found. This genus also has a wide range and many

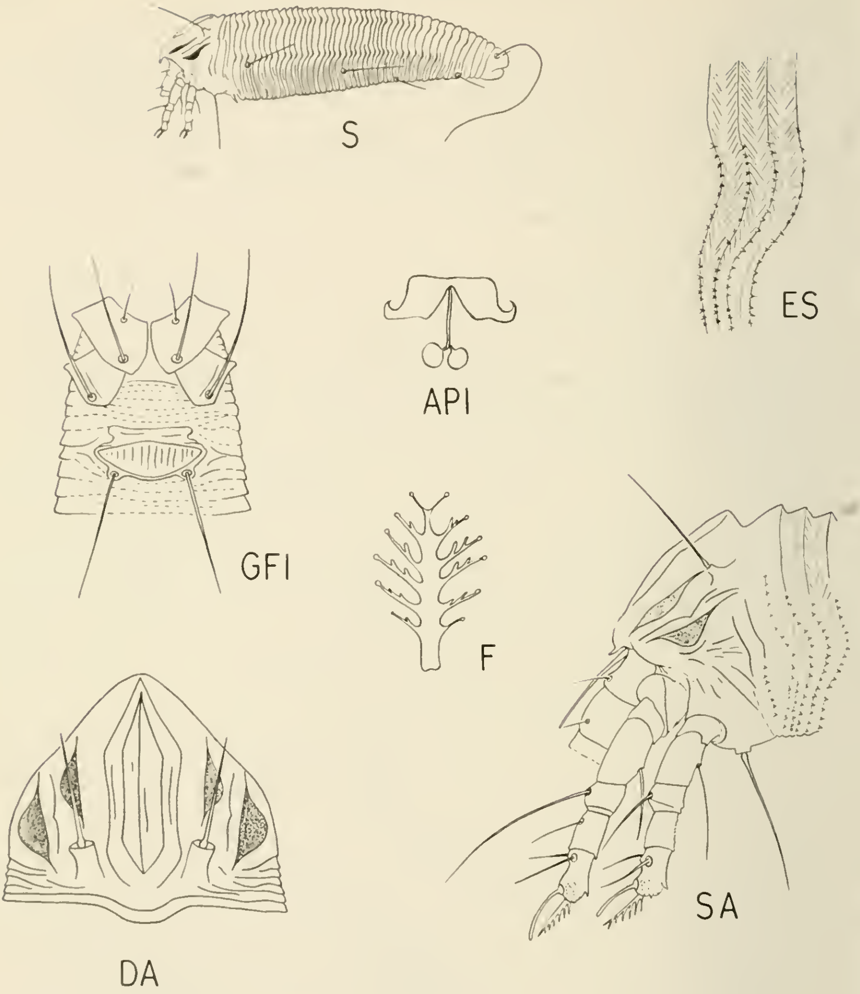


(Plate 17)

species. Nalepa described approximately 50 species from Austria, and Keifer has described several species from California.

Phyllocoptes microspinatus, new species
(Plate 18)

Female: 160 μ long, 38 μ wide, light brown in life, body tapering posteriorly to one-third anterior width. Body usually arched in lateral view.



(Plate 18)

Rostrum 25 μ long, straight, directed downward at slight angle. Chelicerae 15 μ long, practically straight. Shield subtriangular, overhanging rostrum with slight but distinct lip, 27 μ long, 28 μ wide; median line present but not extending entire length of shield; admedian lines present, meeting anteriorly and posteriorly with the median line; submedian lines meeting anteriorly and almost touching posteriorly. Each lateral surface of the shield with two cells somewhat darker than the surrounding area. Dorsal setae 23 μ long, directed anteriorly, usually converging slightly, 16 μ apart at base. Dorsal tubercles 5 μ long. Forelegs 31 μ long; femur 9 μ long, setae 35 μ long; genu 5 μ long, seta 25 μ long; tibia 7 μ long, seta 10 μ long; tarsus 9 μ long, setae

20 μ long. Claw slightly curved, 8 μ long, enlarged slightly at tip. Axis of featherclaw undivided and 6-rayed. Hind legs 28 μ long; femur 8 μ long, seta 15 μ long; genu 4 μ long, seta 15 μ long; tibia 6 μ long, lacking a seta; tarsus 7 μ long, setae 23 μ long. Claw 8 μ long, curved slightly, a little enlargement at the tip. Axis of featherclaw undivided and 6-rayed. Anterior coxae almost twice as large as the hind coxae, setae I and II equidistant from median line. Seta III more laterally placed than setae I and II. Tergites broader than sternites, 53 tergites present. Sternites smaller, microtuberculate with microtubercles present laterally but not on tergites. Microtubercles have very small spines and are located on the posterior margin of the annular ring. Abdomen with four longitudinal ridges, each ridge producing waxy plates and bearing a few spinulate microtubercles. Occasionaly a female specimen is seen which differs only in lacking the four longitudinal ridges and microtubercles; these females are probably deutogynes since they occur in the same population and have all the other characteristics of the species. Genitalia of female 20 μ wide, 12 μ long, about 12 longitudinal scorelines present. Internal apodeme broad, somewhat rectangular. Spermathecae indistinct but apparently round and smaller than usual.

Male: Unknown.

Type locality: Iola, Allen Co., Kansas, Oct. 10, 1954, C. C. Hall.
Type host: *Juglans nigra* L. (Juglandaceae).

Relation to host: Mites were taken from the lower surface of the leaf. There was no obvious damage to the host.

Location of types: Female holotype and two paratype slides so designated from the type locality are deposited in the Snow Entomological Museum, The University of Kansas, Lawrence, Kansas. One paratype slide bearing the same data is deposited in the U.S. National Museum.

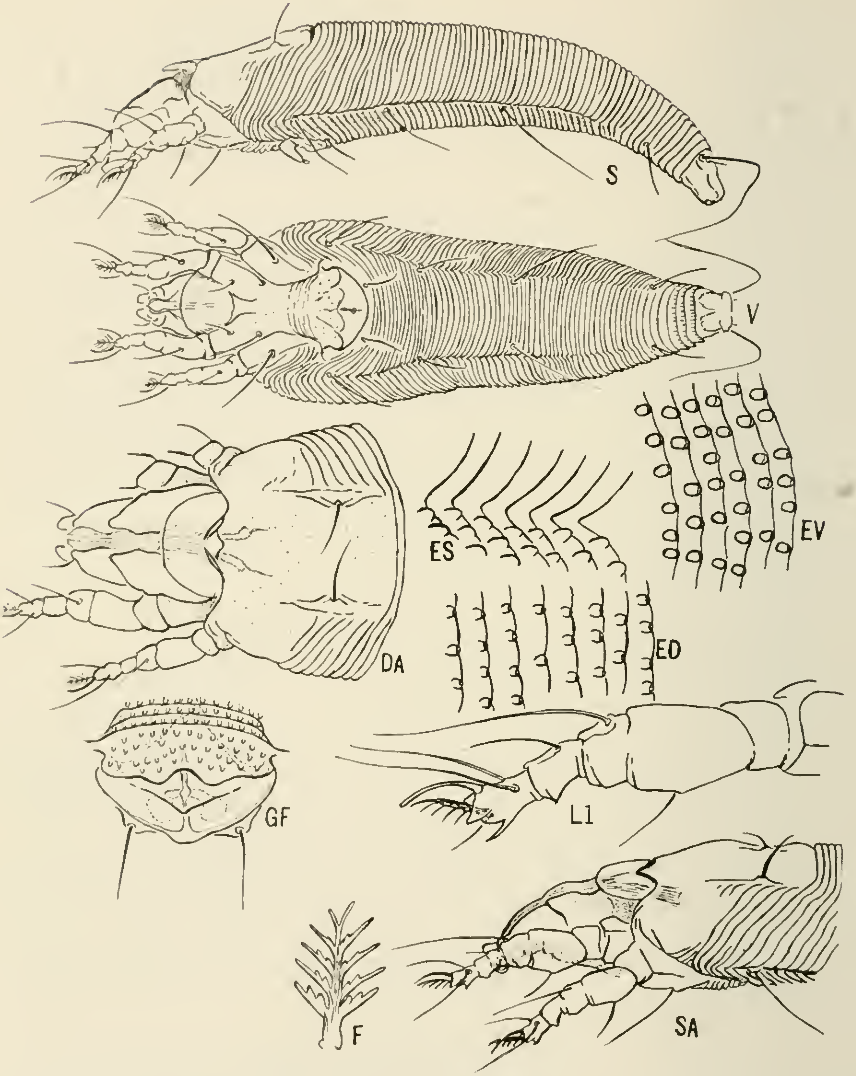
Discussion: This species is similar to *Phyllocoptes adalius* Keifer but is easily distinguished by the shield pattern and lateral markings on the shield of the new species. The posterior margin of the shield is more elevated in the new species than in *Phyllocoptes adalius* Keifer. This last condition is best seen in lateral view.

Genus **Platyphytoptus** Keifer

Platyphytoptus Keifer, 1938, Bull. California Dept. Agr., 27:188.
Type of genus: Platyphytoptus sabinianae Keifer, 1938, Bull. California Dept. Agr., 27:188 (by original designation).

Discussion: Mites of this genus can be recognized by the dorsoventrally flattened body with the abdomen subdivided by a sublateral groove into dorsal and ventral regions. The legs, setae, and featherclaws are not unusual.

Only a few species are described in this genus and all are from California. Material now in my collection indicates that at least one species, *Platyphytoptus sabinianae* occurs throughout the United States.



(Plate 19)

Platyphytoptus sabinianae Keifer

(Plate 19)

Platyphytoptus sabinianae Keifer, 1938, Bull. California Dept. Agr., 27:188.

Type locality: Oroville (Palermo), Butte Co., California.

Type host: *Pinus sabiniana* Douglas.

Additional hosts: *Pinus* spp., including *ponderosa* Douglas, *radiata* Don., *pinea* L., *Torreyana* Parry, *attenuata* Lemm., and *sylvestris* L.

Relation to host: Keifer (1952) states that mites of this species are usually found in needle sheaths living with *Trisetacus pini* (Nalepa) but in Kansas only *Platyphytoptus sabinianae* was found on the host. On another pine in Kansas, *Pinus nigra* Arnold, what appears to be *Trisetacus pini* lives in the absence of *Platyphytoptus sabinianae.* The presence of these mites does not seem to affect the host adversely.

Discussion: This is another example of a species that lives on several different hosts but all within a single genus. Keifer (1952) suggests that for this species to survive on a host, a well formed needle sheath is necessary for the protective niche.

Kansas record: Lawrence, Douglas Co., Sept. 22, 1958, C. C. Hall (from *Pinus sylvestris* L.). This same species was also taken from Christmas trees, *Pinus sylvestris* L., shipped to Kansas from Michigan.

Genus **Mesalox** Keifer

Mesalox Keifer, 1962, Eriophyid studies B-5. Bureau of Entomology, California Dept. Agr., p. 11.

Type of genus: Mesalox tuttlei Keifer.

Discussion: The most distinct characteristics of this genus are the dorsal longitudinal ridge system and the furrow between the ridges. Anteriorly the shield is shaped into a beaklike structure overhanging the rostrum; this is easily seen in lateral view. Divergent dorsal setae are set in dorsolateral tubercles and directed posteriorly. This genus is in the Phyllocoptinae of the Eriophyidae.

Mesalox tuttlei Keifer
(Plate 20)

Mesalox tuttlei Keifer, 1962, Eriophyid studies B-5. Bureau of Entomology, California Dept. Agr., p. 11.

Type locality: Bay City, Michigan.

Type host: Parthenocissus quinquefolia (L.) Planch. (Vitaceae) Virginia creeper.

Relation to host: On Virginia creeper the mites were undersurface leaf vagrants. In Kansas there was no apparent damage to the host (*Vitis* sp., grape) and mites were present on both leaf surfaces.

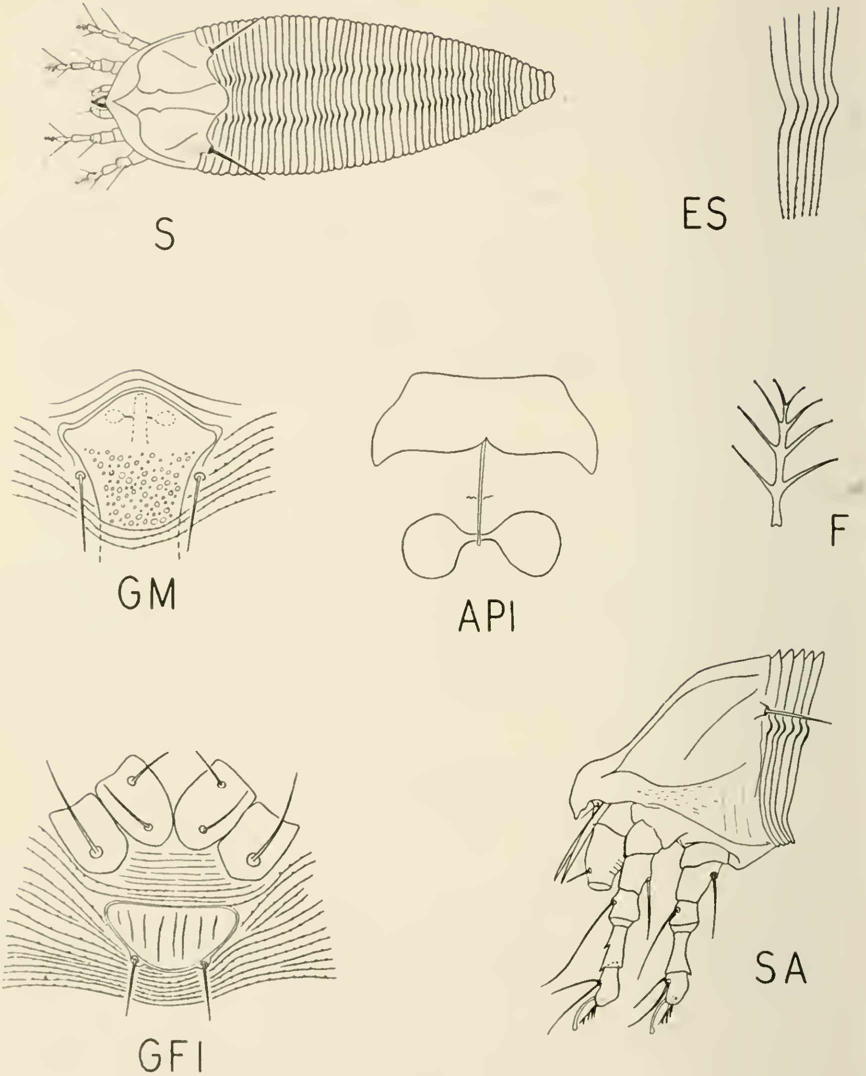
Kansas record: Baldwin, Douglas Co., Kansas, Aug. 11, 1955, C. C. Hall (from *Vitis* sp., Vitaceae, grape). Specimens were not abundant at this time.

Genus **Vasates** Shimer

Vasates Shimer, 1869, Trans. Amer. Ent. Soc., 2:319.

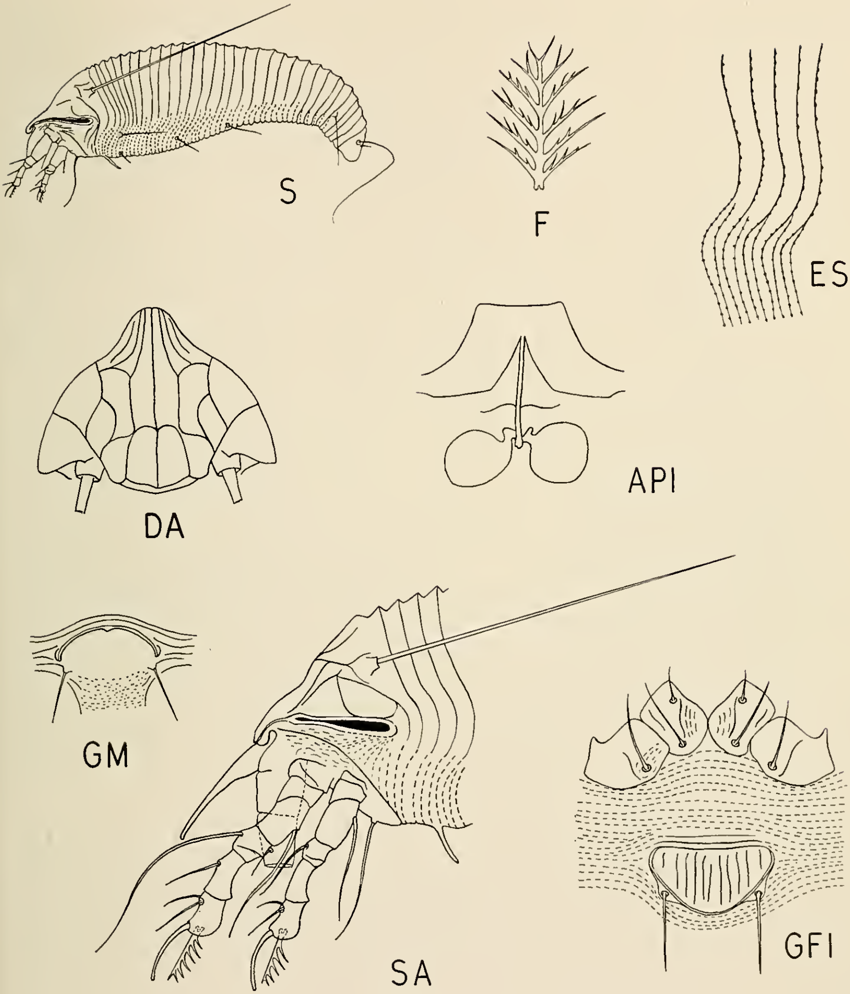
Type of genus: Vasates quadripedes Shimer (by subsequent designation of Keifer, 1944, Bull. California Dept. Agr., 33:25).

Discussion: This genus is characterized by a shield over the rostrum, dorsal tubercles on rear margin of shield, dorsal setae directed upward and caudally converging or diverging, and usually fewer tergites than sternites.



(Plate 20)

Most of the species in this genus are leaf vagrants, causing little or no abnormal growth. There are some exceptions. *V. quadripedes* is a gall-former on maple leaves and injury such as mottling or discoloration of leaves may result occasionally from the feeding of mites in this genus. There are many species that occur commonly in Europe and the United States.



(Plate 21)

Vasates cercidis, new species
(Plate 21)

Female: 170-184 μ long, about 50 μ wide, light brown color, arched strongly in lateral view. Rostrum 20 μ long, evenly curved downward. Chelicerae almost straight, 20 μ long. Shield 32 μ long, 45-55 μ wide, narrowed anteriorly, projected slightly over the rostrum, pattern of irregular cells, median and admedian lines complete, submedian lines present but irregular. Dorsal setae 100 μ long, on conspicuous tubercles, 23 μ apart at base. Forelegs 35 μ long; femur 10 μ long, seta 10 μ long; genu 5 μ long, seta 23 μ long; tibia 7 μ long, seta 6 μ long; tarsus 7 μ long, setae 20 μ long. Claw 8 μ long, curved, without knob at tip. Featherclaw of 6 rays, each ray with 1-3 subdivisions. Hind legs about 30 μ long; femur 10 μ long, seta 10 μ long; genu 5 μ long, seta 10 μ long; tibia 7 μ long, no seta present; tarsus 6 μ long, setae 20 μ long. Claw 8 μ long, curved, without knob at tip. Featherclaw 6-rayed with 1-3 subdivisions on each ray. Anterior coxae slightly contiguous posteriorly, two setae on each; posterior coxae contiguous with anterior coxae, each with a single seta. About 35 tergites and 75 sternites, posterior margins beset with round microtubercles which are more numerous ventrally. Genitalia of female 21 μ wide, coverflap with about eleven longitudinal scorelines. Spermathecae spherical, about 8 μ in diameter.

Male: 165 μ long, 50 μ wide. Genital coverflap 18 μ wide with a shallow median notch on anterior margin, setae 10 μ long.

Type locality: Douglas Co., Kansas, June 12, 1955, C. C. Hall, on University of Kansas campus in front of Snow Hall.
Type host: *Cercis canadensis* L. (Leguminosae).

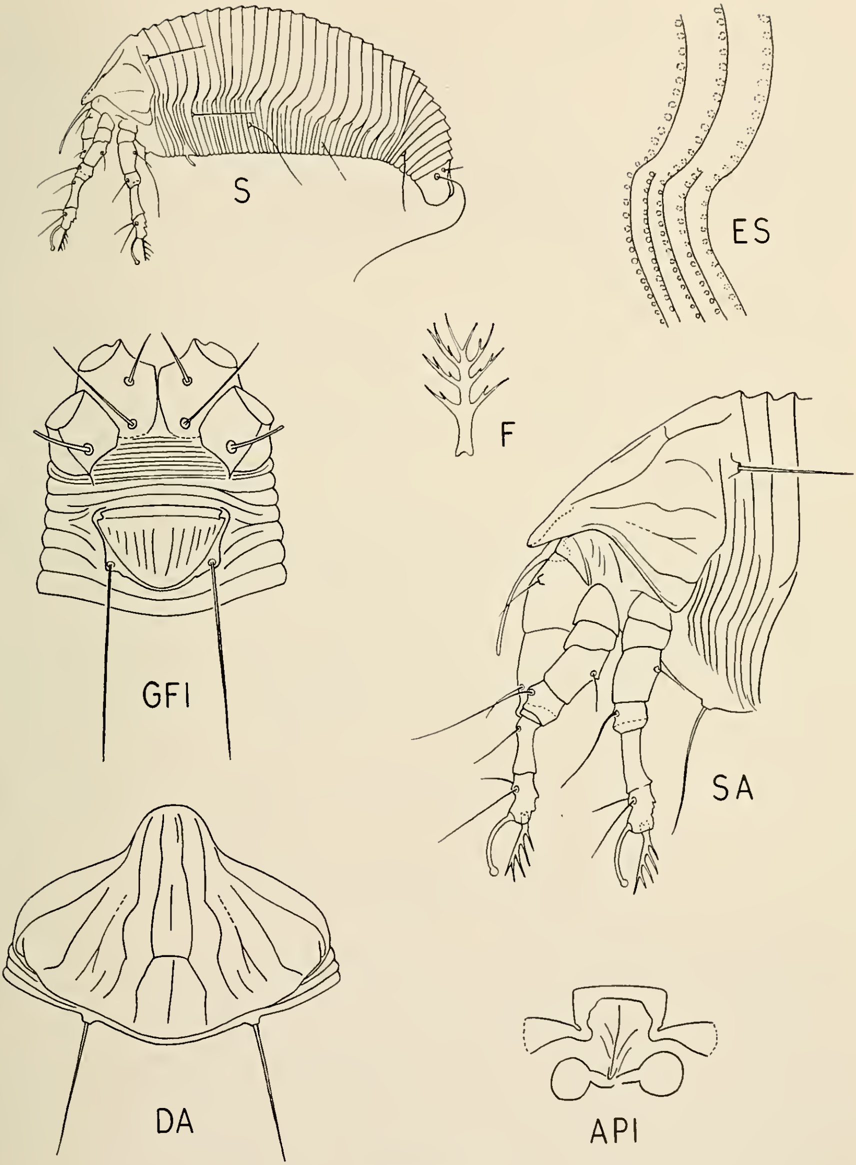
Relation to host: This is a free living species found chiefly on the lower surfaces of the leaves in late summer. For a more detailed account of this species, see the life history section. This species occurs commonly throughout Kansas wherever the host is found. There is no apparent damage to the host.

Location of types: Female holotype, male allotype, and five paratype slides all from the type locality are deposited in the Snow Entomological Museum, The University of Kansas, Lawrence, Kansas. One paratype slide with several specimens sent to the U.S. National Museum.

Discussion: This species is near *Vasates scotti* (Keifer) but can readily be distinguished by the dorsal setae being much shorter in *Vasates scotti*. The shape of the genital coverflap is also useful in separating these two species. In *Vasates cercidis*, the shield bears a lateral, dark, club-shaped mark that is not present in *V. scotti*. The name is formed from the generic name of the host plant.

Vasates dimidiatus, new species
(Plate 22)

Female: 200 μ long, 65 μ wide, dorsum strongly arched, sternites slightly arched in lateral view. Body color light tan in both adults and immatures. Rostrum 28 μ long, with even downward curve. Chelicerae 22 μ long, with slight curve, distal two-thirds of uniform size with slight enlargement at base. Shield 42 μ long, 55 μ wide, anterior lobe rounded and overhanging rostrum. Median line incomplete, admedian lines complete but undulating and diverging posteriorly, submedian lines irregular. Dorsal setae 24 μ long, directed posteriorly from rear shield margin, 35 μ apart, diverging slightly.



(Plate 22)

Dorsal tubercles distinct, about 3 μ long. Forelegs 39 μ long; femur 10 μ long, seta 15 μ long; genu 6 μ long, seta 30 μ long; tibia 12 μ long, seta 8 μ long; tarsus 9 μ long, seta 25 μ long. Claw 7 μ long, arched with knob at tip. Featherclaw 4-rayed with one or two subdivisions on each ray. Hind legs 38 μ long; femur 10 μ long, seta 13 μ long; genu 6 μ long, seta 10 μ long; tibia 9 μ long, lacking a seta; tarsus 8 μ long, seta 25 μ long. Claw 7 μ long, arched with small knob at tip. Featherclaw 4-rayed with small subdivisions. Anterior coxae touching in anterior, medial half. Posterior coxae widely separated, overlapping posterior margin of anterior coxae. All coxae with usual setae. Tergites much wider than sternites, 36 tergites and 72 sternites present. Sternites microtuberculate. Genital coverflap 10 μ long, 25 μ wide, bearing 12 longitudinal scorelines, setae 35 μ long.

Male: Unknown.

Type locality: Lawrence, Douglas Co., Kansas, May 25, 1958, C. C. Hall. Five miles north of Lawrence, Kansas.

Type host: Populus deltoides Marshall (Salicaceae).

Relation to host: There is very little damage to the host: however, the mites do occur more abundantly in marginally rolled leaves and on the lower surfaces of the leaves.

Location of types: A holotype slide and two paratype slides are deposited in Snow Entomological Museum, The University of Kansas, Lawrence, Kansas. Dry leaf samples bearing mummified specimens are in the author's collection.

Discussion: This species is morphologically very similar to *Vasates laevigatae* (Hassan) and *Vasates micheneri.* The genital coverflap and apodemes are the best characters to distinguish this species from *V. micheneri.* The shield pattern and genital coverflap separate this species from *V. laevigatae.* The name of this species is given because the annual rings are divided in half ventrally forming about twice as many sternites as tergites.

Vasates laevigatae (Hassan)
(Plate 23)

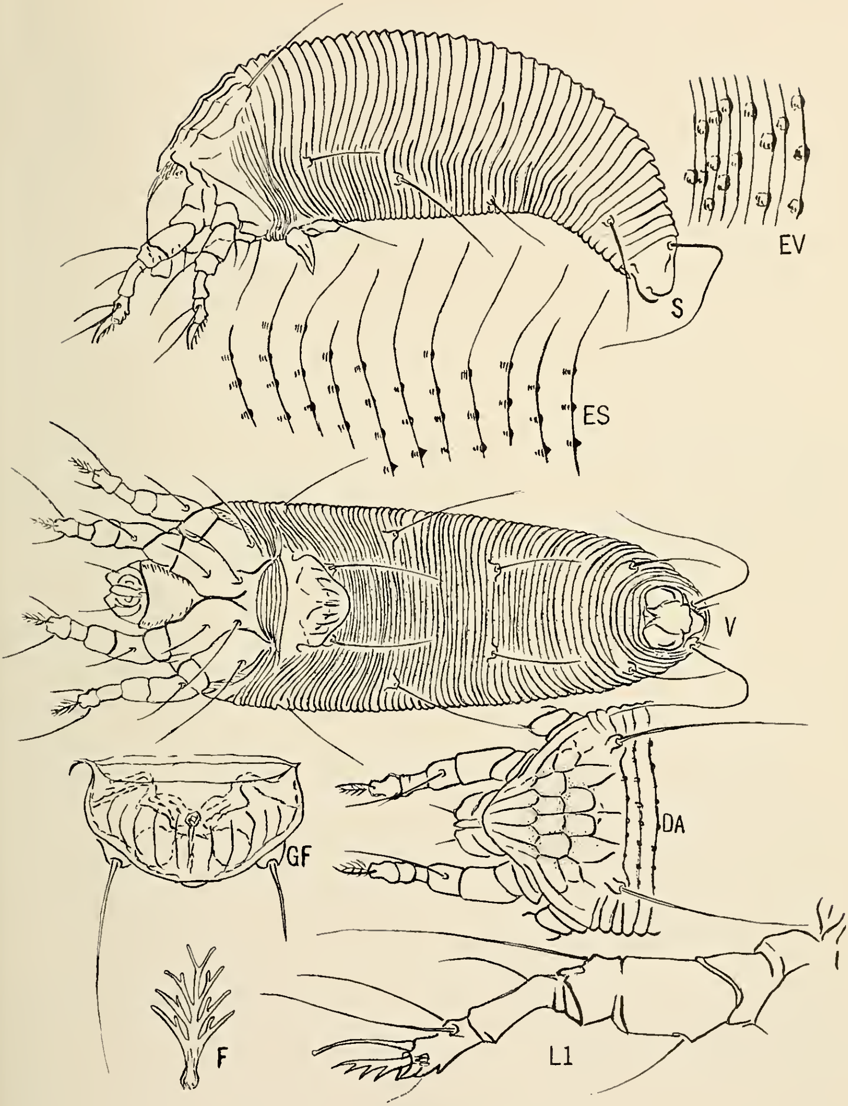
Phyllocoptes laevigatae Hassan, 1928, Univ. California Publ. Ent., 4:379.
Vasates laevigatae (Hassan), Keifer, 1952, Bull. California Insect Survey, 2:45.

Type locality: Agnew, Santa Clara Co., California.

Type host: Salix laevigatae Bebb, red willow.

Relation to host: Keifer reports the formation of beadlike galls on the host and indicates that these galls occur in colonies with some leaves showing many galls while other leaves have none. This same type of gall formation and distribution of galls is typical of this species in Kansas. Serious injury to the host has not been noted.

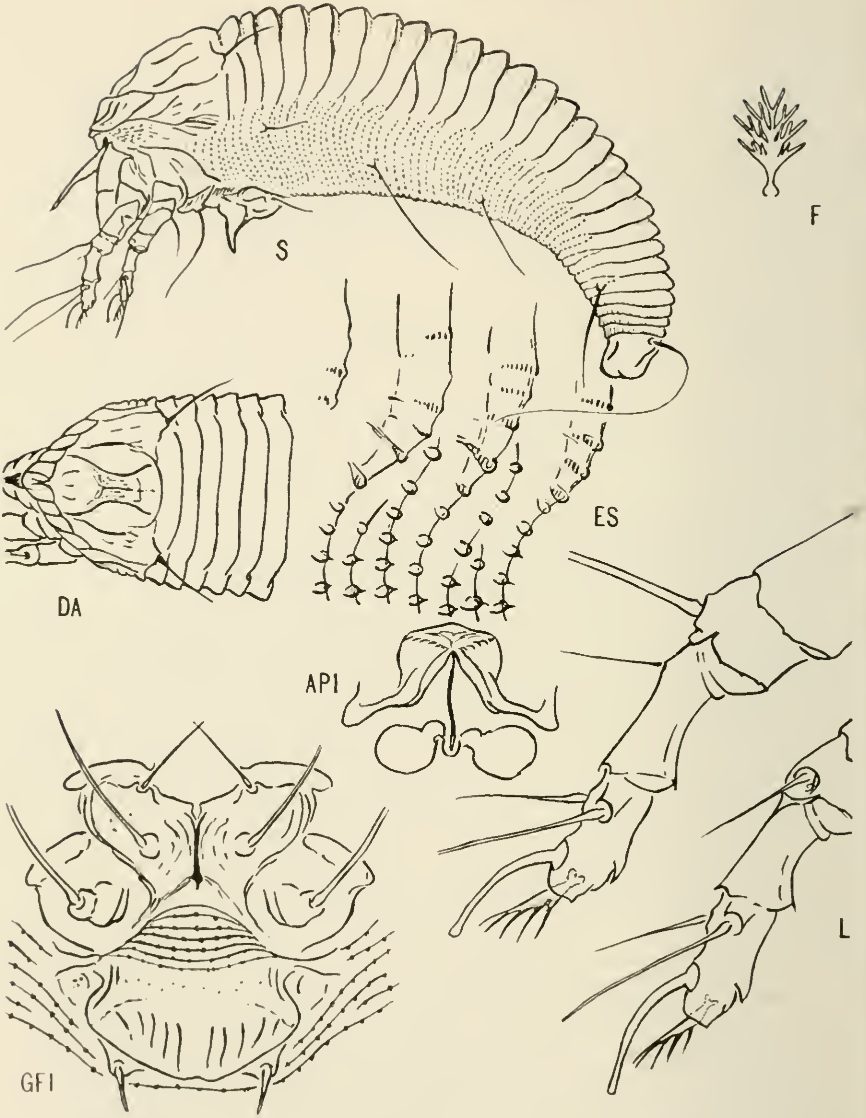
Discussion: Salix seems to serve as a host for several species of eriophyids. Those from willow are difficult taxonomically because some are probably deuterogynous; moreover, the species of willow are not easily determined so that aid from host relations is hard to obtain. These species of eriophyids will probably not be clearly understood until life histories are studied. *Vasates*



(Plate 23)

laevigatae and *Vasates micheneri* are similar species but the genital apodemes are very different; *V. laevigatae* has short, broad apodemes and those of *V. micheneri* are long and narrow.

Kansas record: Lawrence, Douglas Co., on U.S. hwy. 24-40 at east county line, Aug. 27, 1954, C. C. Hall (from *Salix* sp.).



(Plate 24)

Vasates lycopersici (Massee)
(Plate 24)

Phyllocoptes lycopersici Tryon, 1917 *(nomen nudum)*, Rept. Queensland Dept. Agr., p. 53.
Type locality: Australia.
Type host: Tomato.

Phyllocoptes lycopersici Massee, 1937, Bull. Ent. Res., 28:403. *Type locality:* Auckland, New Zealand. *Type host: Solanum lycopersicum* L., tomato.

Phyllocoptes destructor Keifer, 1940, Bull. California Dept. Agr., 29:160. *Type locality:* Modesto, Stanislaus Co., California. *Type host: Lycopersicum esculentum* Miller, tomato.
Vasates destructor (Keifer), Keifer, 1952, Bull. California Insect Survey, 2:44.
Vasates lycopersici (Massee), Lamb, 1953, Bull. Ent. Res., 44:347. *Type locality:* Auckland, New Zealand. Collected by W. Cottier. *Type host: Solanum lycopersicum* L., tomato. Type vial in East Malling Research Station collection, England.

Relation to host: Massee (1937) lists silvering, curling of leaves, leaf drop, blossom drop, and stunted fruits as injuries due to *Vasates lycopersici*. Keifer (1952) states that susceptible varieties of tomatoes are killed by this species. Injury to the host seems to be about the same in Australia, California, Kansas, and New Zealand. Massee did not include a figure in his description of *Phyllocoptes lycopersici*; however, the narrative account and host affinity indicate that *Vasates lycopersici* does occur on tomato in Australia, California, Kansas, and New Zealand. Lamb (1953) gives a complete account of eriophyids occurring on tomato.

Discussion: In his excellent revision of eriophyids occurring on tomato, Lamb (1953) includes convincing evidence supporting the synonymy of *V. destructor* and *P. lycopersici* with *V. lycopersici*.

Kansas record: Galena, Cherokee Co., Dec. 1958, L. A. Calkins (from tomato in greenhouse).

Vasates mckenziei Keifer
(Plate 25)

Vasates mckenziei Keifer, 1944, Bull. California Dept. Agr., 33:26.
Type locality: Sacramento, Sacramento Co., California.
Type host: Elymus triticoides Buckley, a perennial grass.
Additional hosts: Wheat, *Agropyron smithii* Rydb. (western wheatgrass), and *Distichlis spicata* L. (saltmarsh grass).

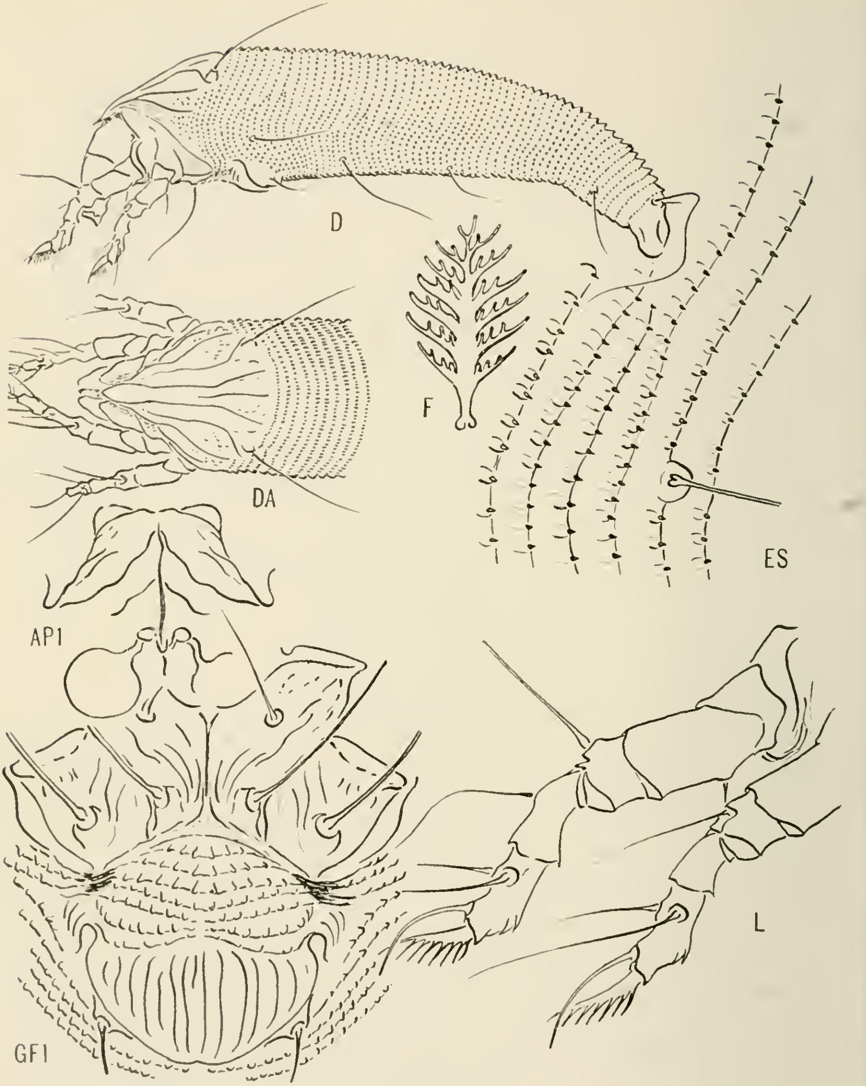
Relation to host: This species has not been reported to cause serious damage to hosts. This species becomes numerous in the furrows on the lower surface of the leaf. Keifer (1952) gives the longitudinal furrows on the upper surface of the leaf as the area in which the mites live on the type host. The occasional occurrence of this species on wheat warrants keeping it in mind as a possible carrier of wheat streak mosaic.

Discussion: In Kansas there is no difficulty in distinguishing this species from other *Vasates*. Keifer, however, indicates the possibility of confusing it with *Vasates dubius* Nalepa. I have not been able to obtain specimens or a drawing of *dubius* but Keifer has stated (correspondence) that one should watch for *dubius* as *mckenziei* is collected and studied.

Kansas records: Hays, Ellis Co., April 21, 1954, T. L. Harvey (from wheat); Hays, Ellis Co., May 7, 1954, T. L. Harvey (from *Agropyron smithii* Rydb., western wheatgrass); Haskell Co., May 17, 1958, J. F. Howell (from *Distichilis spicata* L., saltmarsh grass).

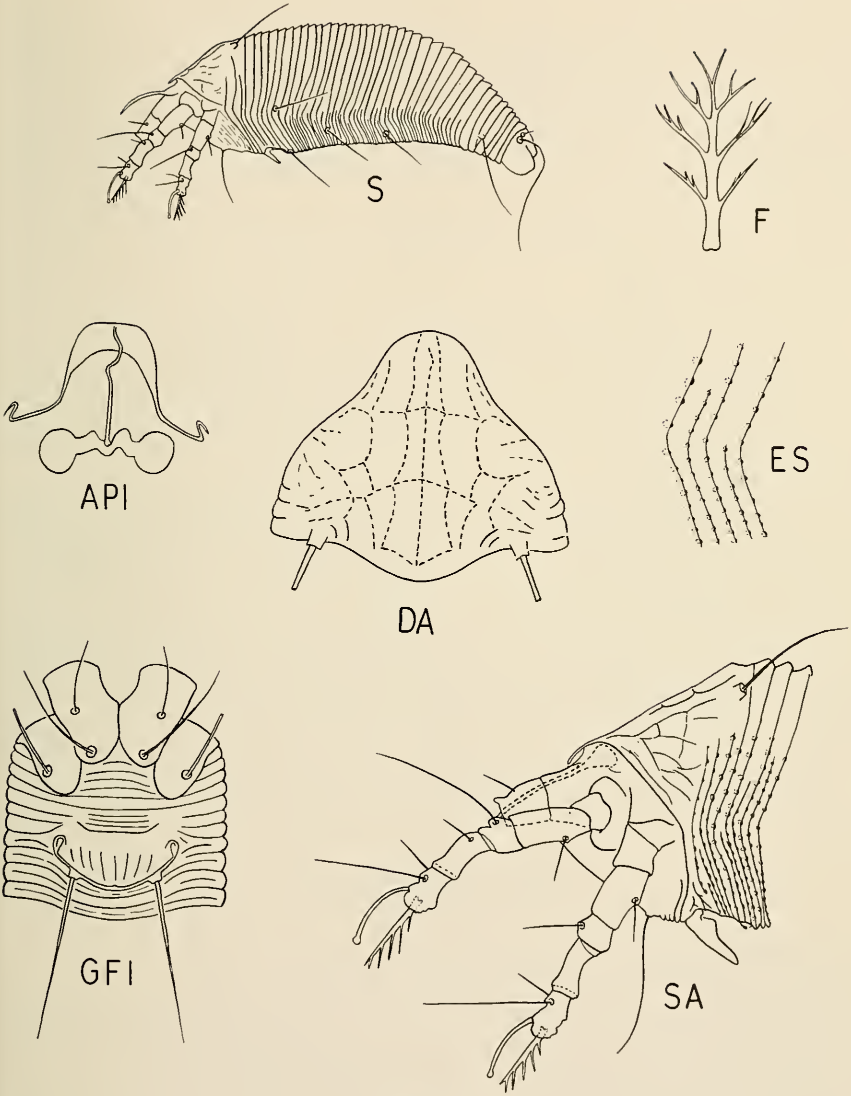
Vasates micheneri, new species
(Plate 26)

Female: 160-190 μ long, 48 μ wide, opaque white in life, slightly arch in lateral view. Rostrum 26 μ long, straight. Chelicerae evenly curved, 23 μ



(Plate 25)

long. Shield 26 μ long, 42 μ wide, subtriangular, extended slightly over the rostrum, rounded anteriorly in dorsal view, shield design weakly expressed consisting of six median cells bordered on each side by three lateral cells. Dorsal setae 33 μ long, directed posteriorly, diverging slightly, 27 μ apart at base. Dorsal tubercles distinct, located on posterior margin of shield. Forelegs 35 μ long; femur 12 μ long, seta 15 μ long; genu 7 μ long, seta 35 μ long;



(Plate 26)

tibia 10 μ long, seta 9 μ long; tarsus 8 μ long, seta 30 μ long. Claw 8 μ long, evenly curved, small knob at tip. Axis of the featherclaw undivided, 4-rayed with small subdivisions. Hind legs 35 μ long; femur 11 μ long, seta 14 μ long; genu 7 μ long, seta 18 μ long; tibia 8 μ long, seta absent; tarsus 8 μ long, seta 25 μ long. Claw 8 μ long, evenly curved, small knob at the tip. Axis of the featherclaw undivided, 4-rayed with subdivisions. Anterior coxae partly touching along medial margins. Posterior coxae contiguous with anterior coxae. Anterior and posterior coxae bearing usual setae. About 60 tergites present; sternites more numerous and microtuberculate. Microtubercles present ventrally and laterally, situated on posterior margin of annular ring. Genital coverflap of the female 20 μ wide, 10 μ long, 12-14 longitudinal scorelines present. Spermathecae 7 μ in diameter, round, connecting ducts about one-half the width of spermathecae.

Male: 218 μ long, 69 μ wide, similar to the female. Males were not common in occurrence.

Type locality: Lawrence, Douglas Co., Kansas, May 25, 1958, C. D. Michener.
Type host: *Salix nigra* Marshall (Salicaceae).

Relation to host: Mites were extremely abundant in distorted flower buds. The infestation had spread to nearly all areas of the tree. Eriophyids on willow are usually in small colonies and do not spread throughout even a single tree.

Location of types: A holotype slide and three paratype slides all from the type locality are deposited in the Snow Entomological Museum, The University of Kansas, Lawrence, Kansas. Dry plant material, containing paratypes is also in the author's collection.

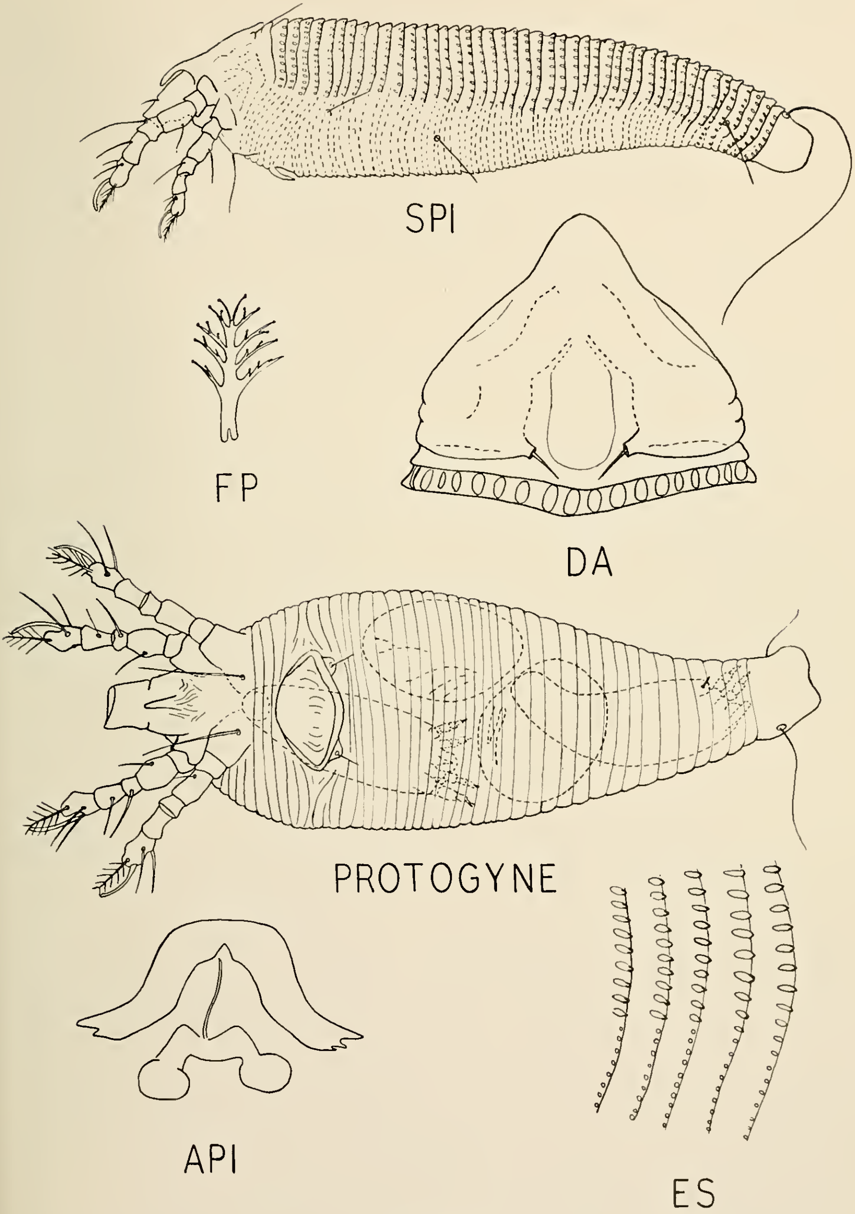
Discussion: This species is very similar to *Vasates rhodensis* Keifer and *Vasates laevigatae* (Hassan). The new species can, on the basis of a 4-rayed featherclaw and genital apodemes, be separated from the two closely related species. *Vasates micheneri* is probably deuterogynous. In some samples either two types of females or two species are present. Careful rearing and seasonal observations must be made to be sure about deuterogyny in this species.

Vasates quadripedes Shimer
(Plates 27, 28)

Vasates quadripedes Shimer, 1869, Trans. Amer. Ent. Soc., 2:319.
Phytoptus quadripes Osborn, 1879, Iowa College Quart., 2:32.
Eriophyes quadripes Banks, 1901, Amer. Econ. Ent., 7:106.
Phyllocoptes quadripes Parrot, Hodgkiss, Schoene, 1906, New York Agr. Exp. Sta. Bull., number 283.
Vasates quadripedes Shimer, Keifer, 1944, California Dept. of Agr. Bull., 33:25.

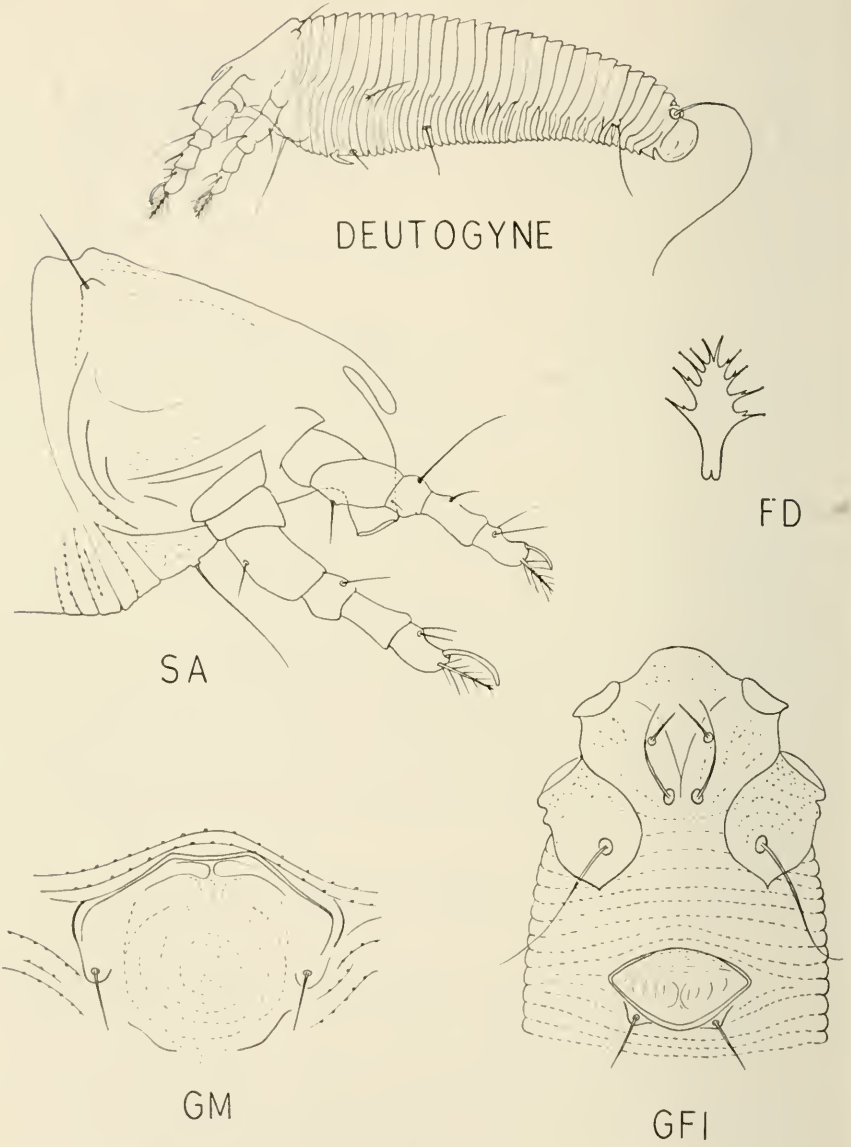
Since the original description and subsequent accounts of this species do not include species characteristics currently being used, they are included here for reference. Figures are also given to help distinguish between the protogyne and deutogyne. Males are noted and a sketch of the male genital coverflap is included.

Female: Protogyne 175-230 μ long, 60-70 μ wide, body round in cross section and wormlike, body pink in color but becoming more red in late summer. Shield 70 μ long, 70 μ wide, tapered anteriorly to a slight projection over the rostrum. Forelegs 42 μ long, all segments present, setae all present.



(Plate 27)

Hindlegs 42 μ long, all segments present, all setae present. Axis of featherclaw 5-rayed with small subdivisions present on some rays. Abdomen



(Plate 28)

composed of about 37 tergites and smaller more numerous sternites, microtubercles present. Genital coverflap with 8 to 10 longitudinal markings present.

Deutogyne 150-175 μ, similar to protogyne in appearance but lacking abdominal microtubercles and longitudinal markings on the genital coverflap. The featherclaw of the deutogyne also is more massive, especially the axis even though it bears the same number of rays.

Male: 110 μ long, 40 μ wide. In some galls males are very common in occurrence. It is not difficult to find males.

Type locality: Mt. Carroll, Illinois.
Type host: Acer dasycarpum, white maple.

Relation to host: The galls produced and resultant damage to the host are adequately described by Shimer (1869) and Hodgkiss (1930). Some of the affected foliage does drop to the ground, especially leaves that are entirely covered with these galls. Shimer (1869) indicates that a thousand or more galls may occur on a single leaf.

Discussion: In addition to the large number of galls produced by this species and resultant damage to the host plant there is an interesting problem concerning the life cycle.

Vasates quadripedes Shimer is undoubtedly deuterogynous. The two types of females and the males are almost always present in samples. I have observed eggs, larvae, and adults in abundance in galls. However, there is some evidence that this species can also be ovoviviparous. I have seen larvae hatched inside the body of the protogyne female (plate 27) and at least one other account of the ovoviviparous habit has been given by Shevtshenko (1961) in his observations on another species *Eriophyes laevis* (Nalepa). Sufficient data are not available now to say more about the ovoviviparous habit, but it does occur in two species of eriophyids.

PHYTOPTIDAE

Genus **Phytoptus** Djuardin

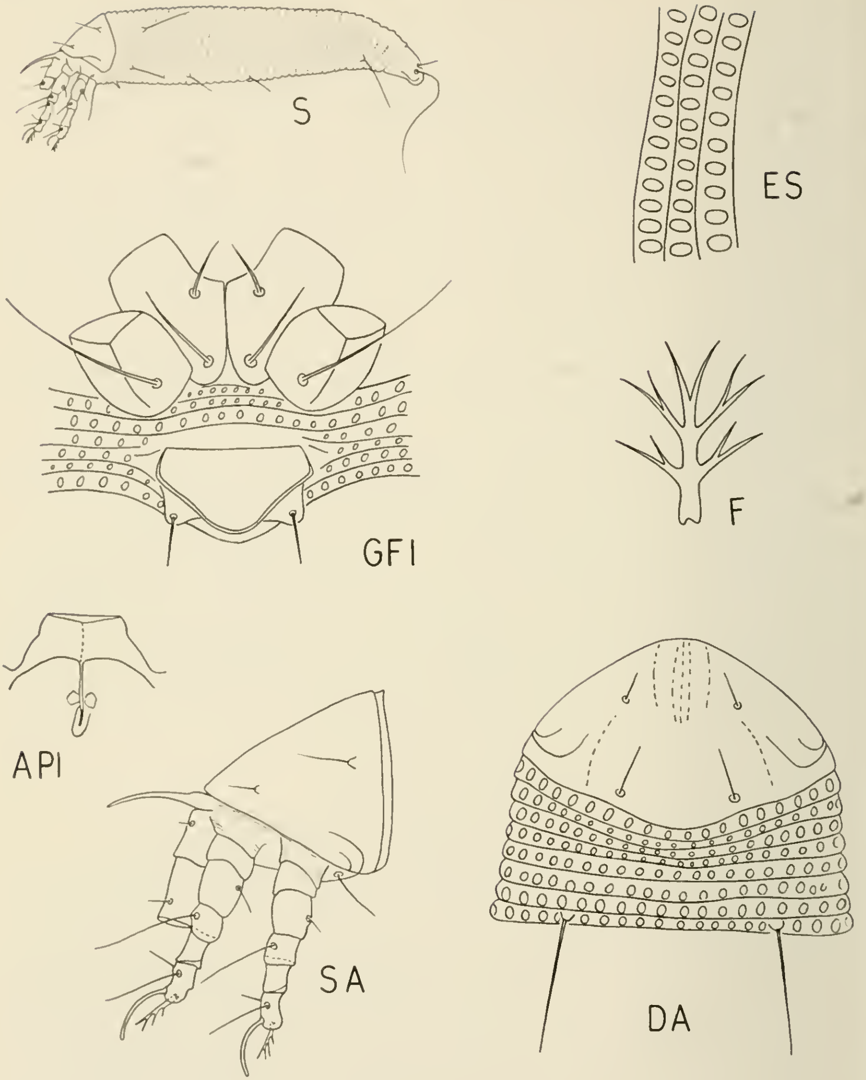
Phytoptus Djuardin, 1851, Ann. Sci. Nat., 15:166.
Type of genus: Phytoptus avellanae Nalepa (by subsequent designation of Keifer, 1938, Bull. California Dept. Agr., 27:301).

Discussion: Only a few genera are known that have four shield setae. Only one, *Phytoptus*, is wormlike, resembling the genus *Eriophyes*, and has subdorsal setae. The shield does not overhang the rostrum in the genus *Phytoptus* and the annular rings are similar above and below; the last seven or eight are wider, both dorsally and ventrally, than the preceding rings.

This genus is now represented by several species, most of which were described by Keifer and Nalepa, chiefly from Austria and the United States.

Phytoptus rotundus, new species
(Plate 29)

Female: 260 μ long, 65 μ wide, wormlike, very little arch to body in lateral view. Rostrum 20 μ long, straight. Chelicerae 15 μ long evenly curved. Shield 32 μ long, 55 μ wide; a few weakly expressed lines in the anterior,



(Plate 29)

central area. Dorsal setae 40 μ long, 20 μ apart at bases. Frontal setae 15 μ long, 18 μ apart at bases. Tubercles of dorsal and frontal setae smaller than average. Forelegs 32 μ long; femur 8 μ long, seta 10 μ long; genu 7 μ long, seta 25 μ long; tibia 7 μ long, seta 5 μ long; tarsus 9 μ long, seta 25 μ long. Claw 8 μ long, curved. Axis of featherclaw undivided, 3-rayed with some subdivision of rays. Hindlegs 32 μ long; femur 10 μ long, seta 10 μ long;

genu 5 μ long, seta 20 μ long; tibia 5 μ long, no seta present; tarsus 9 μ long, seta 25 μ long. Claw 8 μ long, curved, longer than featherclaw. Axis of featherclaw undivided, 3-rayed with some subdivision. Anterior coxae with median margins contiguous. Hind coxae appear to slightly overlay the anterior coxae. Tergites and sternites similar except the last seven or eight annular rings that are wider than the preceding rings. Abdomen bears subdorsal setae 45 μ long, lateral setae 20 μ long, first ventral setae 12 μ long, second ventral setae 11 μ long, third ventral setae 40 μ long, caudal setae, and accessory setae. Microtubercles in side view are ovoid and centrally placed in the annular ring. Genital coverflap of the female without markings, 20 μ wide, 10 μ long. Genital setae 8 μ long. Spermathecae not distinct. Two small dark bodies may be seen adjacent to the posterior extension from the genital apodeme; these are not in the normal position for spermathecae; it is not clear what these structures are.

Male: Similar to female, about 150 μ long.

Type locality: Franklin Co., Kansas, May 15, 1954, R. E. Beer.

Type host: Tilia americana L. (Tiliaceae).

Relation to host: Adult mites, eggs, and immatures were present in small irregular finger galls that occurred on both surfaces of the leaf. The galls were two or three mm in diameter and about four or five mm long. Their color was the same as that of the leaf and the apexes were truncate with several small papillae. Galls were common but other than these the host plant showed no damage.

Location of types: A holotype and five paratype slides bearing type locality data are deposited in Snow Entomological Museum, The University of Kansas, Lawrence, Kansas. One paratype slide with the same data is deposited in the U.S. National Museum. In addition dry paratype material is in the author's collection and dry paratype material has been sent to H. H. Keifer, California Department of Agriculture, Sacramento, California.

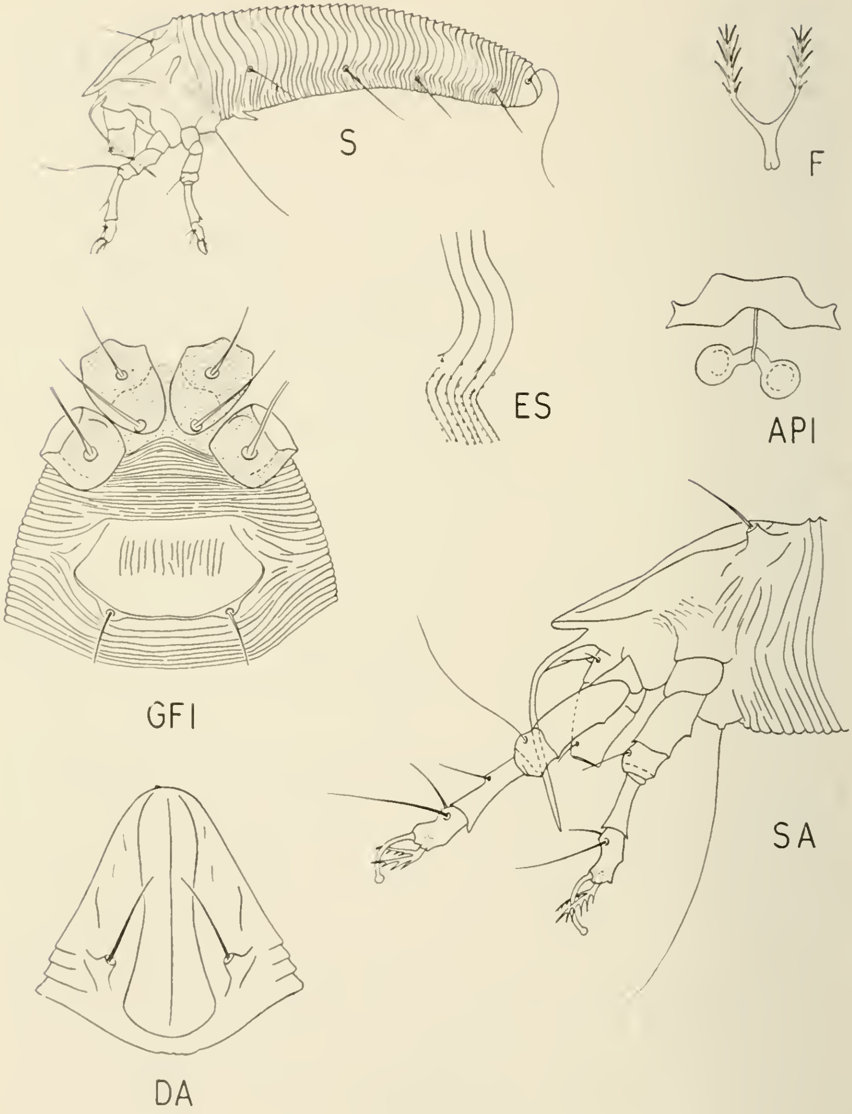
Discussion: Phytoptus rotundus is similar to *P. corniseminis* Keifer but can easily be distinguished by the 3-rayed featherclaw, which seems to distinguish *rotundus* also from other species of the genus. *P. abnormis* Garman is another species that should be noted here. The lack of figures and adequate description make it difficult to determine just what Garman had. The same host plant often does indicate the same species but this is not always true. More specimens are needed to clarify this question.

RHYNCAPHYTOPTIDAE

Genus **Apodiptacus** Keifer

Apodiptacus Keifer, 1960, Eriophyid studies B-1. Bureau of Entomology, California Dept. of Agr., p. 18.

Type of genus: Apodiptacus cordiformis Keifer, 1960, Eriophyid studies B-1. Bureau of Entomology, California Dept. Agr., p. 18.



(Plate 30)

Discussion: Members of the genus *Diptacus* are very similar to *A. cordiformis* K. but the presence of three dorsal longitudinal ridges in the latter easily separates the two genera. Both genera have species capable of producing white wax; in *A. cordiformis* K. white wax stripes are produced along the ridges.

Apodiptacus cordiformis Keifer
(Plate 30)

Apodiptacus cordiformis Keifer, 1960, Eriophyid studies B-1. Bureau of Entomology, California Dept. Agr., p. 18.
Type locality: West Hyattsville, Maryland.
Type host: Carya cordiformis (Wang.) K. (Juglandaceae), bitternut hickory.

Relation to host: No damage is noted to the host and mites are found as tiny tufts of white wax on the lower leaf surface. Material from Kansas had the same appearance.

Kansas record: Baldwin, Douglas Co., Kansas, Aug. 11, 1955, C. C. Hall. Specimens were taken from *Juglans nigra* L. (Juglandaceae) black walnut.

Genus **Rhyncaphytoptus** Keifer

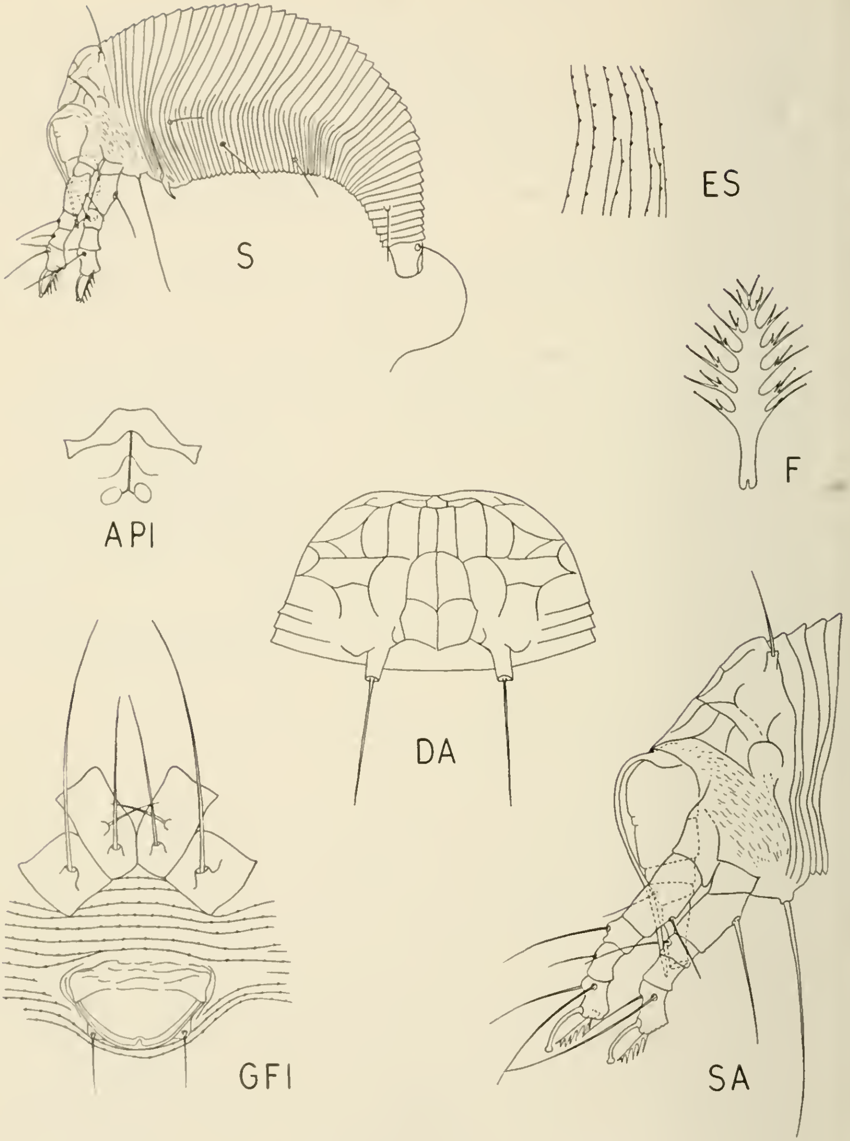
Rhyncaphytoptus Keifer, 1939, Bull. California Dept. Agr., 28:149.
Type of genus: Rhyncaphytoptus ficifoliae Keifer, 1939, Bull. California Dept. Agr., 28:150 (by original description).

Discussion: This genus is characterized by the large, abruptly bent rostrum and chelicerae. Sternites are more numerous than tergites and the axis of the featherclaw is undivided. The last mentioned character is most important and useful in distinguishing this genus from *Diptilomiopus* Nalepa which has a featherclaw with a divided axis.

Little is known about distribution; however, species are common in California and several European species have been reported.

Rhyncaphytoptus boczeki, new species
(Plate 31)

Female: 184-210 μ long, 70 μ wide, robust, semitransparent or very light brown color in life. Body strongly arched in lateral view; posterior third of body tapered somewhat abruptly. Rostrum 40 μ long, projecting down at slight angle. Chelicerae directed anteriorly basally, then strongly bent down, distal fourth slightly recurved. Shield 34 μ long, declivous anteriorly, 55 μ wide. Shield design of irregular cells, 6 cells adjoining median line. Laterally shield appears to have several pronounced ridges that sometimes form cells. Dorsal setae 25 μ long, on tubercles, 17 μ apart at base; directed upward. See lateral view to determine position of dorsal setae. Dorsal tubercles 7-8 μ long, slightly ahead of rear margin of shield, but long enough to extend beyond the posterior shield margin. Forelegs 43 μ long; femur 15 μ long with seta 9 μ long; tarsus 10 μ long, two setae 30 μ long; claw 9 μ long, slightly curved, small knob at tip; axis of featherclaw undivided, 7-rayed, each ray bearing small subbranches. Hind legs 38 μ long; femur 15 μ long on longest side, angular at base, femoral seta 15 μ long; genu 6 μ long bearing a seta 15 μ long; tibia 8 μ long without a seta; tarsus 10 μ long, two setae 30 μ long; claw 9 μ long, curved, small knob at tip; axis of featherclaw undivided with



(Plate 31)

7 rays, rays showing some subdivision. Anterior coxae about twice as long as wide, each coxa bearing two setae, posterior pair of setae about two times length of anterior pair of setae. Posterior coxae almost square, each coxa with a large seta 45 μ long. About 66 tergites; sternites closer together and more

numerous. Microtubercles on rear ring margins, varying from small dots to slightly larger triangles pointed posteriorly. Female genitalia 24 μ wide, setae 10 μ long; spermathecae about 5 μ in diameter, almost round; coverflap with a few basal transverse markings.

Male: Unknown.

Type locality: Kansas City, Wyandotte Co., Kansas, June 9, 1955, by C. C. Hall at Village Specialty Nursery.

Type host: Celtis sp. (Ulmaceae).

Relation to host: The leaves of the host are rough on upper surface and densely pubescent on lower surface. There is no apparent damage to the host. This species was also collected in Douglas Co., Kansas, Aug. 10, 1955, from the same host species.

Location of types: Female holotype and 10 paratype slides all from the type locality are deposited in Snow Entomological Museum, University of Kansas, Lawrence, Kansas. Dry leaves are in the writer's collection. One paratype slide with several specimens sent to U.S. National Museum.

Discussion: This species is fairly close to *Rhyncaphytoptus platani* Keifer but can be distinguished readily by the different shield pattern. It also has the habit of depositing eggs and molting on the tips of plant hairs. Some eggs and molting forms were also observed on the lower leaf surface, but more were seen on the ends of plant hairs.

This species is named for Dr. Jan Boczek, Warsaw Agricultural University, Warsaw, Poland. He showed me what was apparently the same species from *Acer* sp. in Poland and was kind enough to let me describe the species.

Rhyncaphytoptus platani Keifer

(Plate 32)

Rhyncaphytoptus platani Keifer, 1939, Bull. California Dept. Agr., 28:230.

Type locality: Sacramento, Sacramento Co., California.

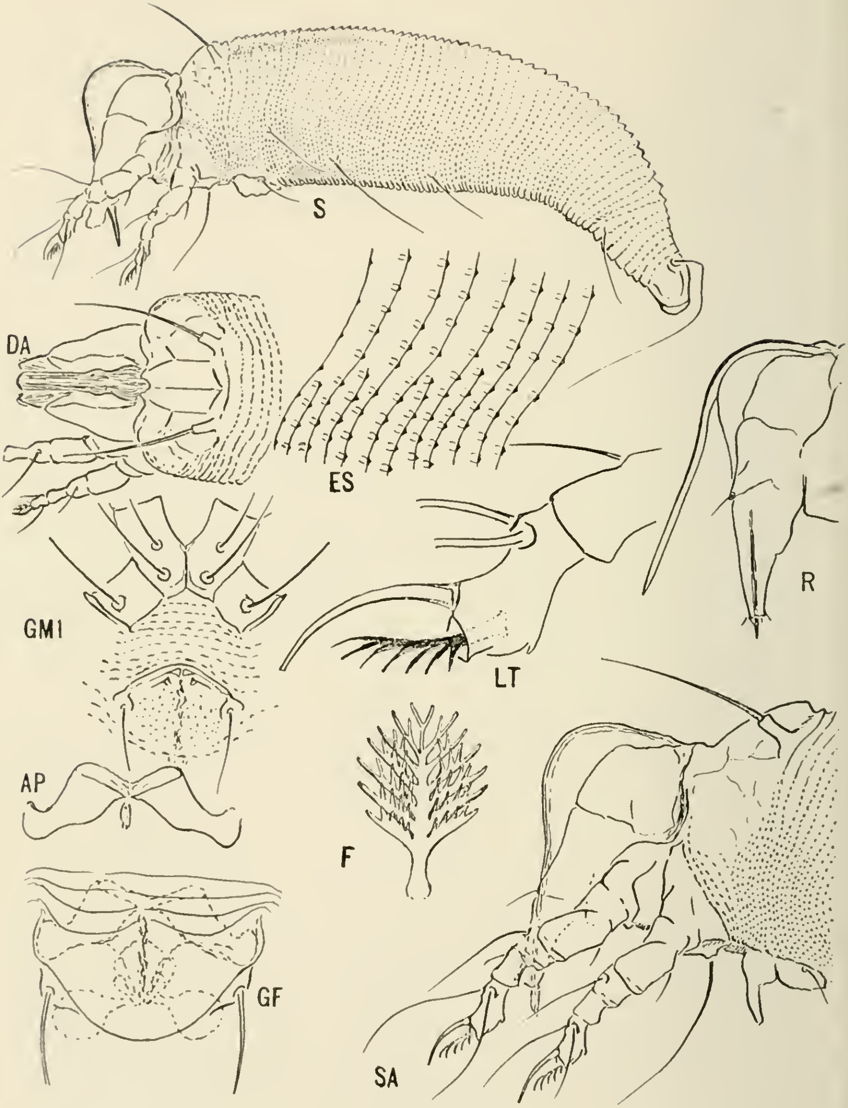
Type host: Platanus sp., a hybrid called "Oriental plane."

Additional hosts: Platanus racemosa Nutt., sycamore and *Platanus occidentalis* L., sycamore.

Relation to host: Mites live on the lower surface of the leaf and cause some browning of leaf tissue. In Kansas this vagrant species only lightly infests the lower surfaces of the leaf and causes no noticeable damage to the host.

Discussion: Rhyncaphytoptus platani and *R. megarostris* (Keifer) look similar. The chelicerae of *platani* seem to be more abruptly bent down than the chelicerae of *megarostris*. The tergites also seem a little wider in *megarostris*. The apodemes, featherclaws, shield pattern, and coverflap are strikingly similar in the two species.

Kansas record: Douglas Co., September 11, 1954, C. C. Hall (from *Platanus occidentalis* L.).



(Plate 32)

HOST LIST

The list below includes mite species associated with each host in Kansas.

Acer saccharinum L. (Aceraceae), white or soft maple

Vasates quadripedes Shimer .... plate 27

Agropyron smithii Rydb. (Gramineae), western wheat grass
    Aceria tulipae (Keifer) .... plate 14
    Vasates mckenziei Keifer .... plate 25
Allium cepa L. (Liliaceae), onion
    Aceria tulipae (Keifer) .... plate 14
Buchloe dactyloides (Nutt.) Engelm. (Gramineae), buffalo grass
    Aceria slykhuisi Hall .... plate 13
Carya sp. (Juglandaceae), hickory
    Aceria erineus (Nalepa) .... plate 8
Carya illinoensis (Wang) K. Koch (Juglandaceae), pecan
    Aceria caryae (Keifer) .... plate 5
Celtis occidentalis L. (Ulmaceae), hackberry
    Aceria celtis (Kendall) .... plate 6
    Rhyncaphytoptus boczeki .... plate 31
Cercis canadensis L. (Leguminosae), redbud
    Vasates cercidis .... plate 21
Cynodon dactylon (L.) Pers. (Gramineae), bermuda grass
    Aceria cynodonis Wilson .... plate 7
Distichlis spicata (L) Greene) (Gramineae), salt marsh grass
    Vasates mckenziei Keifer .... plate 25
Fraxinus americana L. (Oleaceae), ash
    Aceria nimia .... plate 12
Hordeum jubatum L. (Gramineae), barley
    Aceria tulipae (Keifer) .... plate 14
Juglans nigra L. (Juglandaceae), black walnut
    Apodiptacus cordiformis .... plate 30
    Phyllocoptes microspinatus .... plate 18
Medicago satvia L. (Leguminosae), alfalfa
    Aceria medicaginis (Keifer) .... plate 10
Morus sp. (Moraceae), mulberry
    Aceria mori (Keifer) .... plate 11
Morus rubra L. (Moraceae), mulberry
    Aceria lepidosparti Keifer .... plate 9
Opuntia sp. (Cactaceae), cactus
    Aceria cactorum Keifer .... plate 4
Pinus sylvestris L. (Pinaceae), pine
    Platyphytoptus sabinianae Keifer .... plate 19
Platanus occidentalis L. (Platanaceae), sycamore
    Rhyncaphytoptus platani Keifer .... plate 32
Populus deltoides Marsh. (Salicaceae), cottonwood
    Vasates dimidiatus .... plate 22

Salix sp. (Salicaceae), willow	
Eriophyes laevis (Nalepa)	plate 16
Vasates laevigatae (Hassan)	plate 23
Vasates micheneri	plate 26
Solanum lycopersicum L. (Solonaceae), tomato	
Vasates lycopersici (Massee)	plate 24
Sorgum halepense (L). Pers. (Gramineae), Johnson-grass	
Abacarus sporoboli Keifer	plate 2
Tilia americana L. (Tiliaceae)	
Phytoptus rotundus	plate 29
Ulmus americana L. (Ulmaceae), elm	
Aceria parulmi	plate 3
Vitis sp. (Vitaceae), grape	
Mesalox tuttlei	plate 20
Wheat (several varieties)	
Aceria tulipae (Keifer)	plate 14
Yucca glauca Nutt. (Liliaceae)	
Cecidophyopsis hendersoni (Keifer)	plate 15

APPENDIX

MOUNTING MEDIA

Keifer's Solutions (Keifer, 1954)

First Solution:

Resorcinol	50 gms.
Diglycolic acid	20 gms.
Glycerin	25 cc.
Water	10 cc.
Iodine crystals	Enough to produce desired color

Second Solution:

Karo syrup (starch-free)	25 cc.
Chloral hydrate crystals	125 fms.
Glycerin	5 cc.
Water	15 cc.
Iodine crystals	Enough to produce desired color

Third Solution: (Final Medium)

Karo syrup	12 cc.
Chloral hydrate crystals	60 gms.
Potassium iodide crystals	Small amount to keep iodine in solution
Iodine crystals	2 gms.
Formaldehyde solution	Enough to form a thin mixture

CMC-10S—Stain-mountant. Available from Turtox biological supply house, 8200 S. Hoyne Ave., Chicago, Illinois 60620.

Nesbitt's Chloral Hydrate Clearing Solution (Nesbitt, 1945)

Chloral hydrate	40 gms.
Water	25 cc.
Hydrochloric acid	2.5 cc.

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THE UNIVERSITY OF KANSAS
SCIENCE BULLETIN

FINE STRUCTURE OF CRAYFISH OPTIC GANGLIA

By

Richard R. Shivers

Vol. XLVII Pages 677-733 October 11, 1967 No. 10

ANNOUNCEMENT

The *University of Kansas Science Bulletin* (continuation of the *Kansas University Quarterly*) is issued in part at irregular intervals. Each volume contains 300 to 700 pages of reading matter, with necessary illustrations. Exchanges with other institutions and learned societies everywhere are solicited. All *exchanges* should be addressed to

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PUBLICATION DATES

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Vol. XX—October 1, 1932.
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THE UNIVERSITY OF KANSAS
SCIENCE BULLETIN

Vol. XLVII Pages 677-733 October 11, 1967 No. 10

Fine Structure of Crayfish Optic Ganglia[1]

Zoology Department[2]

Richard R. Shivers

ABSTRACT

Whole eyestalks were removed from crayfish and fixed in 6% glutaraldehyde buffered with collidine or phosphate; post-fixation was in buffered 1% OsO_4. Orientation was achieved by examining thick Epon sections with a phase-contrast microscope. Studies with the light microscope were correlated with electron microscopy.

Ganglia are composed of granulated nerve processes, blood sinuses, and glia. Generally, neuronal layers are located at the periphery of the ganglia. Two neurosecretory cell types can be distinguished on the basis of their size and the size of the granules produced. Type 1 neurosecretory cells are most abundant in the X-organ of the medulla terminalis and dense, membrane-limited granules produced in these cells have a diameter of 1000-1700 A. These granules correspond to the major granule type of the sinus gland. The second neurosecretory cell type is located in the neuropil of the medulla terminalis and produces membrane-limited, electron-dense granules which are 1500-2100 A in diameter.

The sinus gland is composed of granulated axons ending near blood sinuses. The most abundant granule type is 1000-1700 A in diameter, whereas a second type has a diameter of 600-1000 A and is not often observed in the sinus gland. The second type is, however, ubiquitous within the ganglionic neuropil.

Many axons associated with synaptic junctions contain both granules and clear vesicles. The granules are 600-1000 A in diameter and the clear vesicles, which are assumed to be synaptic vesicles, are 300-550 A in diameter. Synaptic endings containing only the clear vesicles are also present in the ganglionic neuropil.

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[1] This research was supported by a grant from the University Research Committee to Dr. Paul R. Burton, to whom the author is indebted.

[2] Present address: Zoology Dept., University of Western Ontario, London, Ontario, Canada.

INTRODUCTION

The histological morphology of decapod crustacean optic tracts has been well known since the works of Hanstrom (1928, 1937, 1947, 1953), and an extensive and detailed study of the histology of decapod eyestalks was done by Debaisieux (1944). Neurosecretory cells associated with the eyestalks were shown by Welsh (1941) and Bliss *et al.* (1954) to be located in clusters at the periphery of the optic ganglia. A recent study of the central nervous system of crayfish (*O. virilis* Hagen) (Seabrook and Nesbitt, 1966) elucidated the various nerve tract pathways and the innervations of neuronal beds of the eyestalk.

Since the pioneering works of Scharrer and Scharrer (1940), Bargmann and Scharrer (1951), and Smith (1951), the phenomenon of the production of secretory material in perikarya of specialized neurons and its subsequent transport along axons to areas of storage and release has become widely accepted. This process, called "neurosecretion," has been found to occur not only in the hypothalamo-hypophysial system of higher vertebrates, but also in the medulla terminalis X-organ-sinus gland complex of crustaceans (Bliss *et al.*, 1954; Hanstrom, 1937, 1939; Debaisieux, 1944; Welsh, 1941), the brain-corpus cardiacum-corpus allatum system of insects (Scharrer, 1963), and in other localized systems of nervous elements. Neurosecretion has been found to be an integral factor in the function of many invertebrate nervous systems (Scharrer, 1963, Hagadorn *et al.*, 1963; Fingerman and Aoto, 1959, Gray *et al.*, 1964; Coggeshall, 1965; Eakin and Westfall, 1965; Lentz and Barrnett, 1965, Morita and Best, 1965; 1966; Boer, 1965; Thomsen, 1965; Normann, 1965; Vollrath, 1966; Johnson, 1966; King *et al.*, 1966; Schreiner, 1966, Hsiao and Fraenkel, 1966; Messner, 1966; Bowers and Johnson, 1966, Block *et al.*, 1966; Lane, 1966; Simpson *et al.*, 1966).

Although much work has been done on neurosecretion with the light microscope, it has only been in the past several years that neurosecretory systems have been studied with the electron microscope. The studies by Scharrer (1963), Hagadorn *et al.* (1963), Morita and Best (1965, 1966), and Block *et al.* (1966), were among the first to demonstrate the production of discrete neurosecretory products in the form of electron-dense, membrane-limited granules. Problems involving the mechanisms of release of these secretory materials have recently been investigated with the electron microscope (Weiss, 1965; Johnson, 1966), and several authors have reviewed what is known of the action of neurosecretions (Knowles, 1965; Scharrer, B., 1965; Scharrer, E., 1965).

The sinus gland, a neurohaemal organ of the crustacean eyestalk, has been studied with the electron microscope by Hodge and Chapman (1958), but their micrographs were of such high magnification that the general fine structure of this gland was not shown. There is no ultrastructural evidence to indicate the mechanism of release of secretory products from the sinus gland, although such activity would certainly be expected in such an active endocrine gland.

With the exception of a few investigators (Hamori and Horridge, 1966a, 1966b, 1966c, 1966d), the crustacean eyestalk has been virtually ignored by electron microscopists, perhaps due to its great complexity. Hamori and Horridge studied the fine structure of the optic lamina of the lobster, which corresponds to the lamina ganglionaris of the crayfish optic tract. The ultrastructure of crayfish rhabdomeres and retinular cells has been studied by Eguchi (1965), and Fingerman and Aoto (1959) examined the neurosecretory system of dwarf crayfish with the electron microscope. Fingerman and Aoto's research contained only superficial observations of the optic ganglia and they reported that neurosecretory granules were not membrane-limited.

Probably the most thoroughly investigated physiological activity of crayfish eyestalks has been that of the endocrinological control of the migration of both retinal and chromatophore pigments (Scharrer, 1941; Brown, 1944, 1951; Welsh, 1941, 1951; Kleinholz, 1942, 1961, 1966; Carlisle and Knowles, 1959; Fingerman and Lowe, 1957; Fingerman, 1957, 1965a, 1965b, 1966). Eyestalk substances have definite endocrinological activities in the control of various physiological phenomena in Crustacea. According to Kleinholz (1966), such phenomena include: (1) ovary size, (2) blood glucose levels, (3) molting, (4) retinal pigment migration, (5) erythrophore pigment movement, and (6) melanophore pigment movement. Pasano (1953) correctly showed the source of a molt-inhibiting compound to be in the medulla terminalis of the optic tract and he related this substance to the sinus gland and to the X-organ. Kleinholz (1966) discussed the separation and purification of the eyestalk hormones and he noted that the active substances of the eyestalk are of a proteinaceous nature, some of which are low molecular weight polypeptides.

Cyclical activity of the production of neurosecretory substances in the eyestalk has been investigated by Enami (1951a, 1951b) and Webb (1966). Pyle (1943) presented evidence for cyclical activity in the sinus glands of several species of crustaceans, but he could show no cyclical activity in the cells of the X-organ.

The purpose of this paper is to correlate light and electron microscopic studies of crayfish optic ganglia. Also, neurosecretory components of the eyestalk will be described and related to information on hormonal activities associated with the optic tract.

MATERIALS AND METHODS

Adult crayfish *(Orconectes nais)* were obtained from ponds at The University of Kansas Fisheries Laboratories, Lawrence, Kansas. Animals were considered to be adult if the cephalothorax length measured greater than 33 mm. Crayfish were kept in covered aquaria filled with 5-6 cm of pond water at about 20°C. Crayfish were fed larvae of *Tenebrio molitor.* The laboratory was lighted artificially from 8 o'clock A.M. to 6 o'clock P.M. and also for occasional periods during the evening. Only light-adapted animals were fixed and no discrimination was made between sexes. Entire eyestalks were removed by severing the optic nerve proximal to the exoskeletal covering of the eyestalk. The eyestalks were placed in a drop of cold fixative and the exoskeleton was split longitudinally with iridectomy scissors to allow penetration of the fixative. The exoskeleton was completely removed later in the fixation process. Most eyestalks were embedded whole, but in some cases they were dissected into individual ganglia before embedding.

Light Microscopy. Eyestalks were removed and placed in either Bouin's Fixative or in 10% formalin. The material was fixed for 10 days at room temperature and then dehydrated in a series of graded alcohols and xylene. The tissue was embedded in 56-58° C. M.P. paraffin and serial sections were cut at 6-7.5 μ. Sections were mounted on 1" × 3" glass microscope slides and series were stained with one of the following solutions: (1) Harris' Hematoxylin and counter-stained with Eosin, (2) acetic thionine for Nissl substance (McManus, 1960), (3) chrome-alum hematoxylin (Gomori, 1941), (4) iron-alum hematoxylin and fast green, and (5) Ramon y Cajal pyridine-silver. Light micrographs were made on 4" × 5" Kodak Panatomic-X Professional Sheet Film attached to a Zeiss phase-contrast microscope.

Electron Microscopy. Tissues were fixed for up to 2 hours in one of the following solutions (at 4° C.):

1. 6% glutaraldehyde (Sabatini *et al.*, 1963) buffered with s-Collidine (Bennett and Luft, 1959) at pH 7.6-7.75, to which had been added sucrose (0.045 gm./ml. fixative according to Caulfield, 1957) and $CaCl_2$ (10^{-3}M).
2. 6% glutaraldehyde buffered with 0.5 M sodium phosphate at pH 7.45-7.75 (sucrose and $CaCl_2$ added as above).
3. 1% paraformaldehye and 25% glutaraldehyde (Karnovsky, 1965) buffered with 0.5 M sodium phosphate at pH 7.5 (sucrose and $CaCl_2$ added as above).

Following primary fixation, eyestalks were washed in the appropriate buffer solution for 1-1½ hours during which time the exoskeleton was removed from the eyestalk. Postfixation was for 1 hour in 1% osmium tetroxide buffered as with the primary fixative. The only exception was following the use of Karnovsky's fixative when the osmium tetroxide was buffered with s-Collidine at pH 7.8. Following fixation, material was dehydrated in an ascending series of graded alcohols to propylene oxide, and embedded in Epon 812 (Luft, 1961).

Silver or gray sections were cut with glass knives on a Porter-Blum MT 1 Ultramicrotome and picked up on 150 and 200-mesh copper grids which were coated with a thin film of Parlodion and a thin layer of amorphous carbon. Sections were stained either with lead citrate (Reynolds, 1963) or with both lead citrate and a saturated aqueous solution of uranyl acetate.

Negative staining was accomplished by placing a freshly dissected medulla terminalis in a drop of 1% sodium phosphotungstate (Parsons, 1963). The tissue was teased apart in the solution with fine dissecting needles and drops of the resultant solution were placed on 150-mesh copper grids coated with Parlodion and allowed to dry.

Ultrastructural studies were made with an RCA EMU 3-H electron microscope operating at 50 kv. with a 35-40 μ aperture. Micrographs were made on Kodak Projector Slide Plates at initial magnifications of about 1,800 to 29,000 diameters and were further enlarged photographically using Schneider Companon Lenses. For purposes of measurement, the microscope was calibrated with two different ruled carbon grating replicas.

Correlation of sections for light and electron microscopy was achieved by cutting 1 μ thick Epon sections and either mounting them in glycerol on glass microscope slides for examination with phase-contrast optics, or staining thick sections, mounted dry on glass microscope slides, with methylene blue and azure II (Richardson *et al.*, 1960) for bright field microscopy. When a particular area had been identified with the light microscope, the parent Epon block could be trimmed accordingly and thin sections obtained of that area for electron microscopy. To save time, thick sections were occasionally cut free-hand with a razor blade and mounted in glycerol.

List of Abbreviations

AL—Axolemma
AP—Adhaerence plaque
AX—Axon
BC—Blood cell
BL—Basal lamina (external lamina)
BM—Basement membrane
BS—Blood sinus
CE—Optic chiasma externis
CI—Optic chiasma internis
CSG—Mature dense body
CT—Connective tissue
ER—Endoplasmic reticulum
F—Fibroblast
G—Golgi body
GL—Glial cell
GLY—Glycogen
H—Haemolymph
L—Large granule
LG—Lamina ganglionaris
LY—Lysosome
M—Mitochondrion
ME—Medulla externis
MI—Medulla internis
MT—Medulla terminalis
N—Nucleus
NEU—Neuron
NP—Nuclear pore
NSC1—Type 1 neurosecretory cell
NSC2—Type 2 neurosecretory cell
NT—Neurotubule
NU—Nucleolus
OB—Onion body
ON—Optic nerve
P—Neuropil
PC—Sinusoidal supporting cell
PM—Plasma membrane
R—Rhabdomere
S—Small granule
SG—Sinus gland
SV—Synaptic vesicle
SY—Synapse
T—Microtubule
XO—X-organ

OBSERVATIONS

Light Microscopy

The crayfish optic tract consists of three well-defined ganglia and a fourth less well-defined ganglion known as the lamina ganglionaris. The ganglia are arranged in a consecutive manner behind the eye, and the last ganglion tapers to form the optic nerve which passes to the brain (Fig. 1). The ommatidia and retinula cells with their rhabdomeres rest upon a dense fibrous layer known as the basement membrane through which pass axons of the retinula cells.

Groups of neuron cell bodies are located at the periphery of the ganglia although they may be found within the ganglionic neuropil. The major constituents of the ganglia are neuropil, blood sinuses, blood cells, and glia. Large cells possessing one or two very prominent nucleoli (Figs. 22, 23) can be seen in an area between the medulla externis and medulla internis and also along the periphery of the fourth ganglion, which is the medulla terminalis. These cells, designated here as type 1 neurosecretory cells, are characterized by an extremely large nucleus (12-16 μ in dia.) and relatively scant cytoplasm (Figs. 21-23). Most of these cells are grouped in an area on the anterior tip of the medulla terminalis between the medulla internis and the medulla terminalis. This area is known as the X-organ (Figs. 1A, area 8; 22, 23). Processes of these cells (type 1 neurosecretory cells) can be seen passing into the neuropil of the medulla terminalis (Figs. 22, 23) and are assumed to ultimately end in the sinus gland (Figs. 15-18). These neurosecretory cells are mixed with glial cells and neurons (Figs. 21-23). With the

FIG. 1. Light micrograph montage of the eyestalk. Acetic thionine, phase-contrast. ×90. NOTE: Electron micrographs are of material fixed in collidine-buffered glutaraldehyde unless otherwise indicated.

light microscope, no apparent association between neurons and neurosecretory cells can be detected. Numerous mitochondria can be seen in the neurosecretory cells and in the axons of the ganglionic neuropil (Figs. 22, 23).

The sinus gland, a highly vascularized area in which granulated axons terminate, is located in an area peripheral to the optic tract between the medulla externis and the medulla internis (Fig. 1). Two optic chiasmata are present, one between the lamina ganglionaris and the medulla externis, and a second which is located between the medulla externis and the medulla internis (Figs. 1, 14). The chiasmata are areas in which nerve fibers from several areas of a ganglion cross and enter the following ganglion at a site opposite from their point of origin. The optic nerve originates as a part of the medulla terminalis and passes posteriorly to the brain. It is composed of axonal bundles made up of varying numbers of axons and also of glial cells which apparently provide an insulational sheath for the axons (Fig. 1), a point that will be discussed later.

Only 4 cell types can be distinguished in the eyestalk with the light microscope. Type 1 neurosecretory cells can be readily identified by their large size and prominent nucleoli. The neuronal cell bodies found in groups at the periphery of the ganglia cannot be subdivided into types. With the light microscope they all appear morphologically homogeneous and much smaller than the neurosecretory cells. Glial cells cannot be visually distinguished from neurons, but some distinction can be made on the basis of location, inasmuch as glial cells are usually located within the neuropil of the ganglia. Cells believed to be white blood cells are easily distinguished in sinuses both within and without the ganglia. The area of the eyestalk which is anterior to the lamina ganglionaris and posterior to the proximal pigmented area is called the zona fasciculata (after Debaisieux, 1944) (Fig. 1). Blood cells (arrow) can be seen floating free in the large sinusoidal areas surrounding the retinular axons passing posteriorly through the basement membrane. The sinus gland of Fig. 1 is seen as a highly vascular area containing projections from the medulla externis, medulla internis, and the internal optic chiasma, and the vascular portion of the gland is seen to contain numerous blood cells (arrows). Blood sinuses and lacunae are also seen within the ganglionic neuropil. The medulla terminalis in Fig. 1 is seen to contain various dense structures, one of which can be seen at the arrow, which are blood sinuses that often contain blood cells. As will be shown later, these sinuses are quite complex.

Figure 1A is designed to provide a means whereby electron micrographs can be correlated with an overall view of the entire eyestalk as seen with the light microscope. The electron micrographs are representative of blocked areas of Fig. 1A. The explanation of Fig. 1A indicates which electron micrographs are associated with each numbered area.

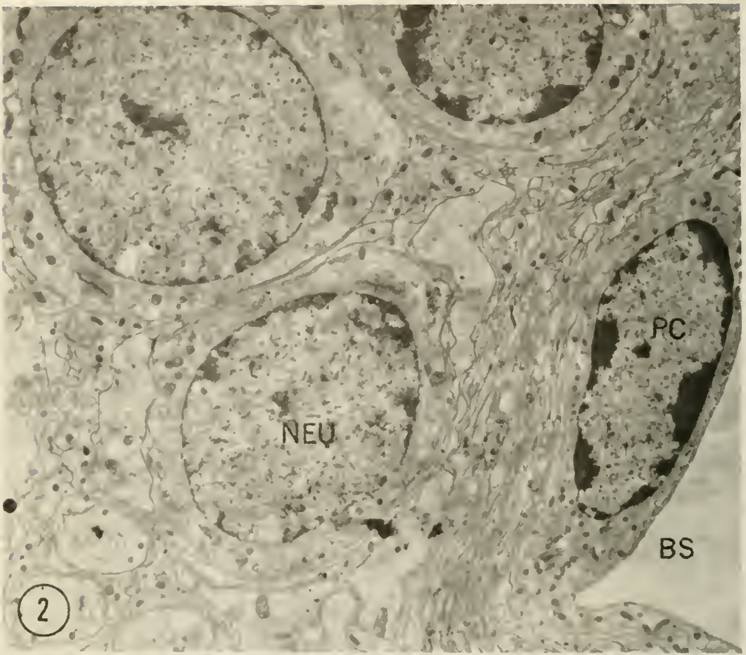
FIG. 1A. Light micrograph montage of the eyestalk. Acetic thionine, phase-contrast. ×90. Numbered areas of this figure correspond to electron micrograph figures as follows: Area 1: Figs. 2, 3, 4, 5. Area 2: Figs. 6, 7. Area 3: Figs. 19, 20. Area 4: Fig. 14. Area 5: Figs. 15, 16, 17, 18. Area 6: Figs. 8, 9, 10, 11, 12, 13. Area 7: Figs. 24, 26-33. Area 8: Figs. 21, 22, 23, 25, 34, 35, 36. Area 9: Fig. 50.

ELECTRON MICROSCOPY

The area numbered 1 of Fig. 1A is a region composed of large bundles of axons originating from retinular cells. There is not a 1:1 correspondence between processes passing through this area and the retinula cells; each axonal bundle is composed of axons from several retinular cells. In the terminal portions of these axonal bundles, before they enter the lamina ganglionaris, a neuronal associational area is present which is characterized by the presence of many neuronal cell bodies. This area can be seen in the lower portion of area 1 of Fig. 1A. Figures 3 and 4 show numerous neurons found in this associational area. Large blood sinuses are extremely abundant and are surrounded by a "sinusoidal supporting cell" (Fig. 2). The arrangement of neuronal processes around the "sinusoidal supporting cell" and its sinus seen in Figs. 2, 5, and 10 should be noted. This axonal arrangement is common to this area of the eyestalk and is frequently seen associated with vascular entities in the neuropil. These areas are characterized by the disposition of axonal processes in concentric circles around blood sinuses (Figs. 5, 10), and such axons are smaller in diameter than those of the ganglionic neuropil (Fig. 5).

The four optic ganglia of the crayfish eyestalk appear structurally similar when seen with the electron microscope. Random thin sections of the four ganglia exhibit many features common to all of the ganglia (Figs. 6, 7, 8, 9, 19, 24, 26, 29, 30, 32). All ganglia contain blood sinuses, glial cells, granulated axons, synaptic areas, large nerve tracts, and occasionally, neuron cell bodies. For the most part, neuron cell bodies are located in layers at the periphery of each ganglion and also between each ganglion. Since all ganglia are structurally similar, identification of the ganglion in question is necessary prior to electron microscopic studies. The medulla terminalis is the only ganglion which contains structures not common to the other three ganglia. This ganglion contains cells which make up the X-organ, which will be discussed later.

Neuropil of the medulla terminalis contains a cell type designated as type 2 neurosecretory cells (Figs. 31, 32). Although not abundant, these cells seem to be restricted to the medulla terminalis. Their nuclei closely resemble those of neurons of the ganglionic neuropil. Rough endoplasmic reticulum (reticular membranes studded with ribosomes) is located almost exclusively around and near the nucleus (Figs. 31, 33), and the reticulum is usually seen as a few elongate membranes associated with clusters of free ribosomes (Fig. 33). Few mitochondria are seen in this cell type and the inset of Fig. 31 shows a high magnification of an unusual "crystalline" mitochondrion seen here. A Golgi body is usually present (Fig. 33) and is associated with both clear and dense-centered vesicles. The cytoplasm of type 2 neurosecretory cells is filled with electron-dense, membrane-limitd granules (1500-2100 A in dia.) which



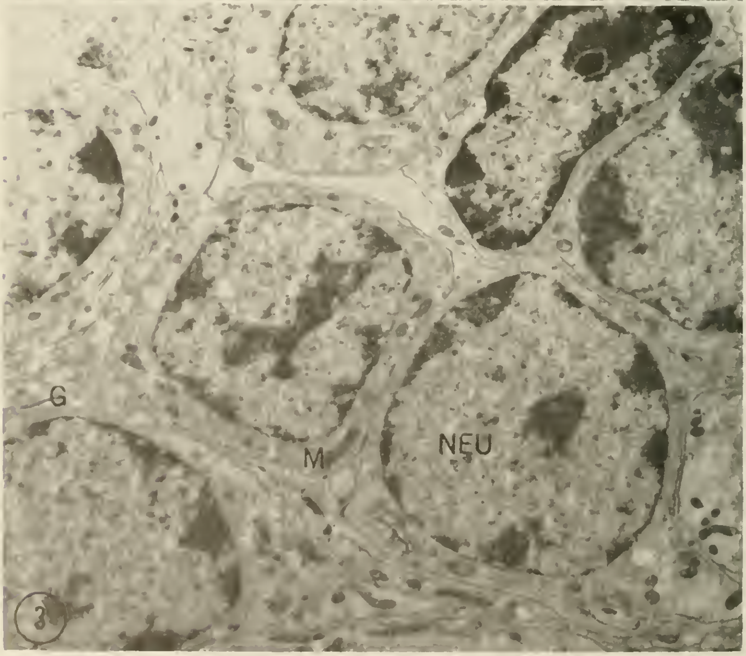
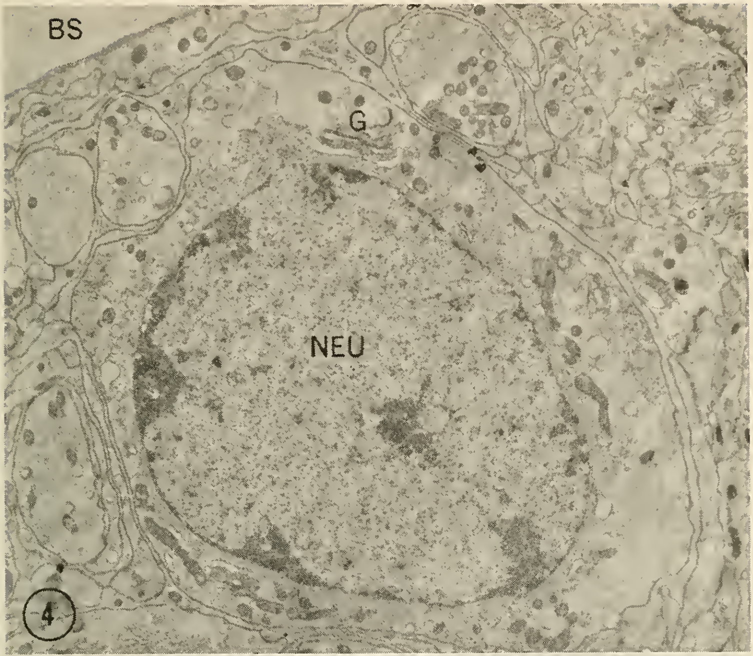


FIG. 2. Zone fasciculata. Neurons near a blood sinus. LC; ×3,704. FIG. 3. Zona fasciculata. Neuron perikarya. LC; ×4,760.



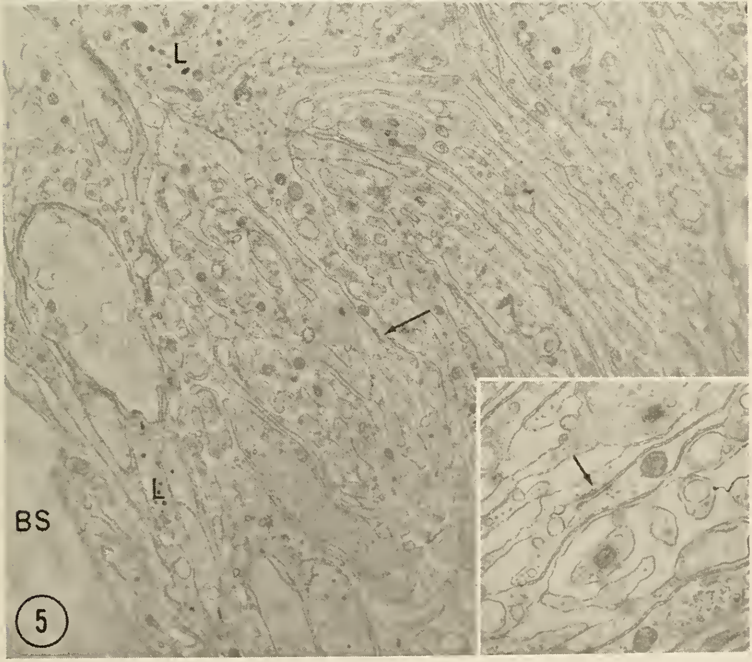


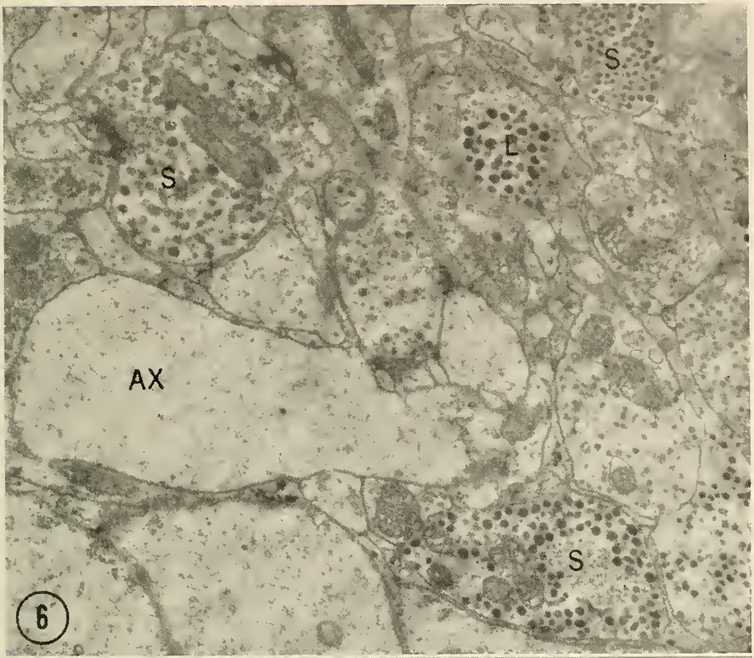
Fig. 4. Zona fasciculata. Neuron cell body located near a blood sinus. LC; ×4,760. Fig. 5. Zona fasciculata. Annulate axonal bed surrounding a blood sinus. Note the adhaerence plaques (arrows). LC; ×5,863. Inset; ×13,029.

possess a very small electronlucent "halo" between the granule membrane and the dense core. Occasionally some of these granules can be seen near the rough endoplasmic reticulum in what may represent a stage in their release by the endoplasmic reticulum (Fig. 33). Large bodies filled with a dense and heterogeneous material are present in the cytoplasm and are assumed to be lysosomes (LY). Processes of these cells have not been observed elsewhere in the ganglion or eyestalk.

Neuron Cell Bodies. All neuron cell bodies, with the exception of type 1 neurosecretory cells, are morphologically similar when seen with the light microscope. With the electron microscope however, distinct structural differences can be seen. Neurons of the zona fasciculata and of the peripheral cell layers contain rounded nuclei with varying amounts and distributions of dense chromatin material. The neurons in Figs. 2 and 4 are about the same size as those in Fig. 3. However, the nuclei of those in Fig. 3 exhibit more dense chromatin near the nuclear envelope than do those in Figs. 2 and 4. Numerous elongate mitochondria are present in these cells and Golgi bodies are occasionally seen (Figs. 2-4). Extensive arrays of rough endoplasmic reticulum are absent. Instead, the rough endoplasmic reticulum occurs as small vesicles (Figs. 2-4). Most ribosomes in such cells are not associated with membranes but appear in the form of clusters (polyribosomes). Large numbers of polyribosomes give a dense, particulate appearance to the cytoplasm of the neuronal perikarya (Figs. 2, 3, 4, 27). No secretion bodies or structures such as lysosomes can be seen in the neuronal perikaryon.

Neuron cell bodies located within the ganglionic neuropil exhibit the same characteristics as do the peripherally located neurons described above, with the exception of nuclear morphology and the appearance of the rough endoplasmic reticulum. The nuclei of these intra-ganglionic neurons exhibit an irregular shape with numerous indentations of the nuclear envelope (Figs. 10, 26). Dense chromatin is seen near the nuclear envelope (Figs. 10, 26). The rough endoplasmic reticulum is not organized into groups of lamellae but is found as isolated lengths of membrane (Figs. 10, 26, 27). Ribosomes are also found clustered in the cytoplasm. Neurotubules measuring about 250 A in diameter are infrequently seen in the cytoplasm of the cell bodies and usually occur as relatively short structures randomly oriented in the cytoplasm (Fig. 27). No secretion bodies can be seen in the perikaryon, but occasionally structures resembling lysosomes are present.

Structures designated as adhaerence plaques are occasionally seen between plasma membranes of neurons and their processes (Figs. 5, 25, 27, 28). In the lower left of Fig. 27 are two structures assumed to be adhaerence plaques, although they do not exhibit some of the features commonly associated with desmosomes. The opposing plasma membranes of the two neurons appear to be somewhat thickened since they are more electron-dense than in other



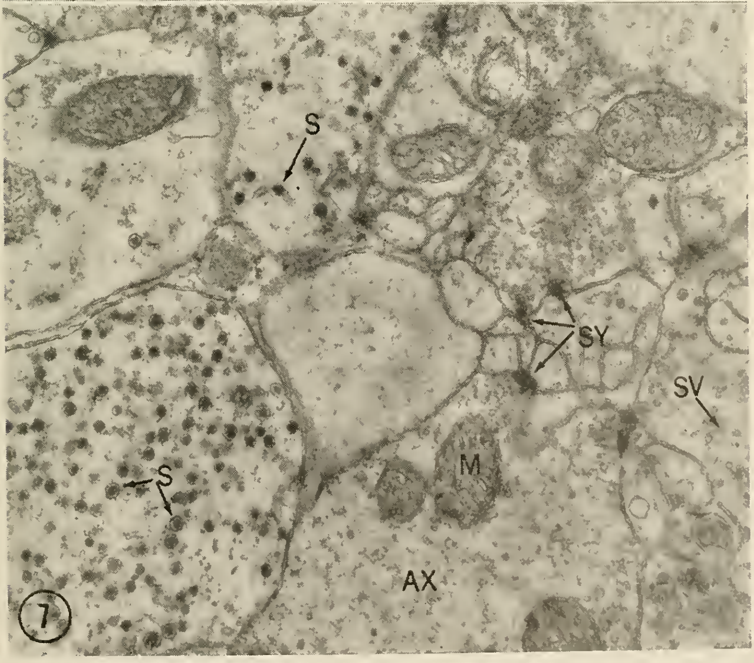
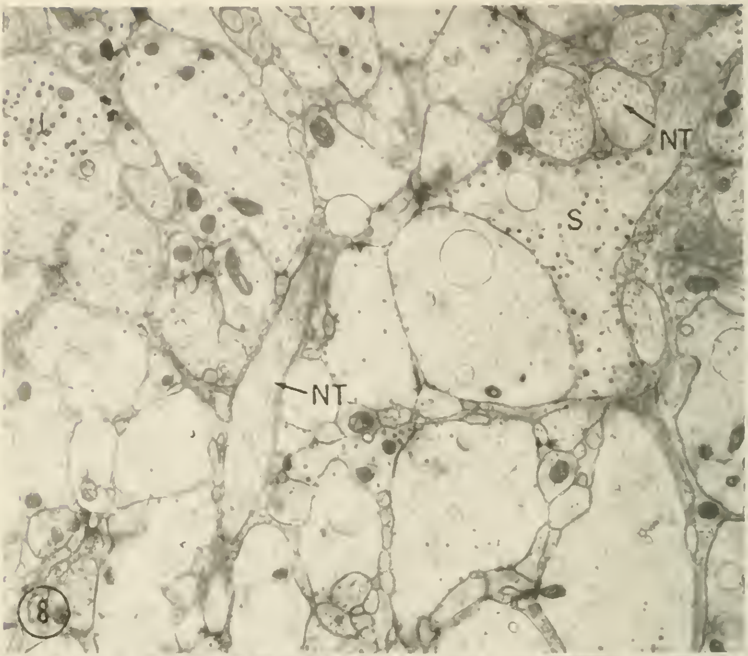


Fig. 6. Lamina ganglionaris. Field containing many granulated nerve processes. LC-UA; ×16,416. Fig. 7. Lamina ganglionaris. Note the numerous synaptic boutons containing only clear vesicles. LC-UA; ×21,287.



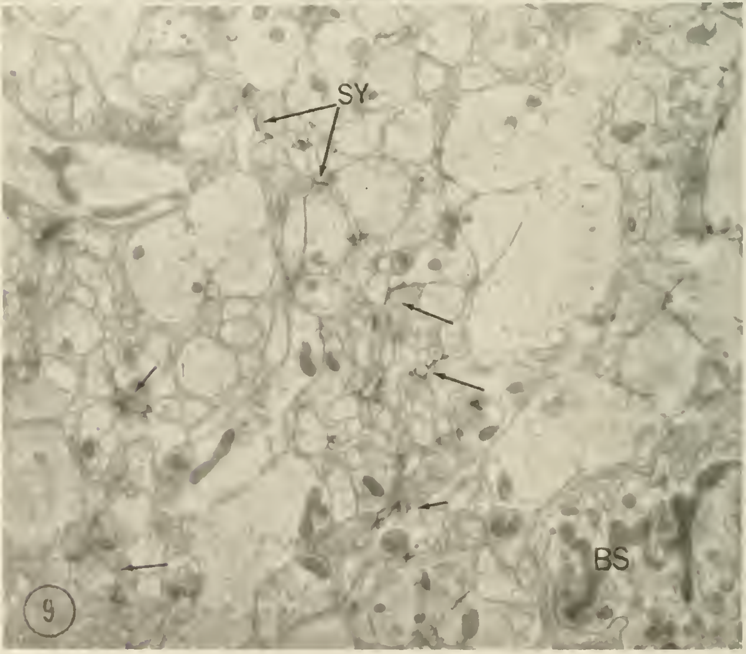


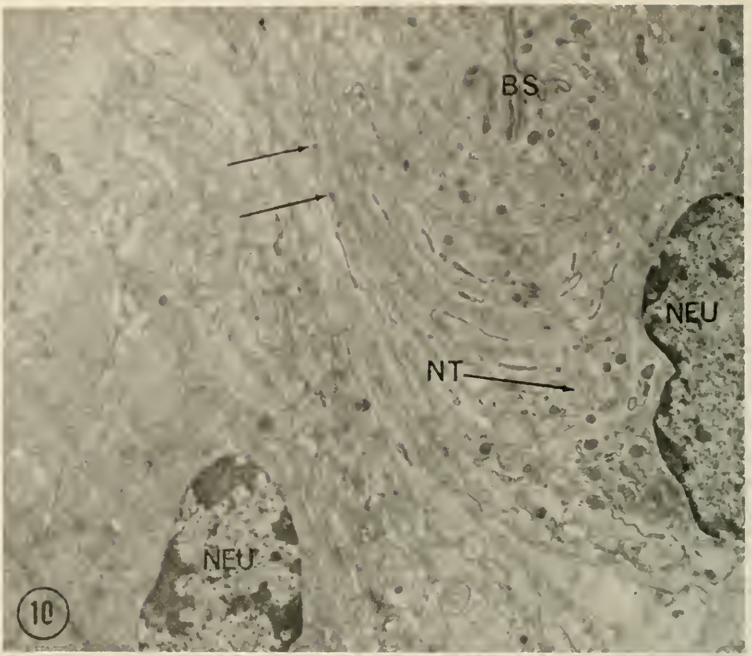
FIG. 8. Medulla internis. General survey micrograph. LC-UA; ×8,686. FIG. 9. Medulla internis. General survey micrograph. Note the synaptic junctions at the arrows. LC-UA; ×5,906.

areas of the plasma membrane. The other adhaerence plaques can be seen at high magnification in Fig. 28. These plaques are associated with the opposing membranes of two neuronal processes and the membranes are separated by a space of about 190 A—a flocculent, non-fibrous material is located between the membranes and in the neuronal cytoplasm just beneath the plasma membranes.

Neuronal Processes. The ganglionic neuropil is mainly composed of granulated and non-granulated neuronal processes. General survey electron micrographs of such processes can be seen in Figs. 6, 7, 8, 9, 19, 24, 29, 30, and 32. Two general classes of neuronal processes can be distinguished in the ganglionic neuropil (Figs. 6, 7, 8, 9, 19): (1) granulated axons, and (2) non-granulated axons. All neuronal processes seen in the optic ganglia possess small mitochondria, typically located just beneath the plasma membrane. Axonal neurotubules are often seen and are generally oriented parallel to the long axis of the axon (Figs. 8, 12, 20, 28, 29). An unusual relationship is seen in Fig. 28. A single neurotubule is apparently continuous with rough endoplasmic reticulum (arrow); the cisterna of this reticular membrane is thus continuous with the lumen of the neurotubule.

Axonal granules can be divided into two classes according to their size: (1) electron-dense, membrane-limited granules which are 1000-1700 A in diameter which are identified by "L" (large granule), and (2) electron-dense, membrane-limited granules 600-1000 A in diameter which are identified by "S" (small granules). Clear vesicles 300-550 A in diameter are frequently present in axonal endings and are assumed to be synaptic vesicles. Small granules (S) exhibit a rather prominent "halo" which represents the clear space between the dense core of the granule and its limiting membrane (Fig. 38). Large granules (L) also possess such a "halo" but it is much less prominent (Figs. 37, 29, 41, 21). The large granules occasionally appear crystalline with repeating cross-bands measuring about 50 A wide; such dense bands are separated from one another by spaces about 90 A wide (Fig. 39). The crystalline structure of these granules is not often seen, and they usually appear as membrane-limited, dense, granules with a particulate central core (Figs. 37, 41, 42).

Axons frequently contain both clear vesicles (300-550 A in dia.) and small secretory granules (Fig. 6). Small dense secretion granules are ubiquitous throughout the ganglionic neuropil whereas the large granules are rarely seen within axons of the neuropil. Large secretion granules comprise the major granule type in the sinus gland and will be discussed later. Granulated axons always contain granules of one size class and "mixing" of large and small granules within the same axon is never seen (Figs. 6, 7, 24, 29, 30, 32). Large granules have occasionally been observed outside axons containing similar granules (Fig. 29 and inset). This condition is not common and may reflect



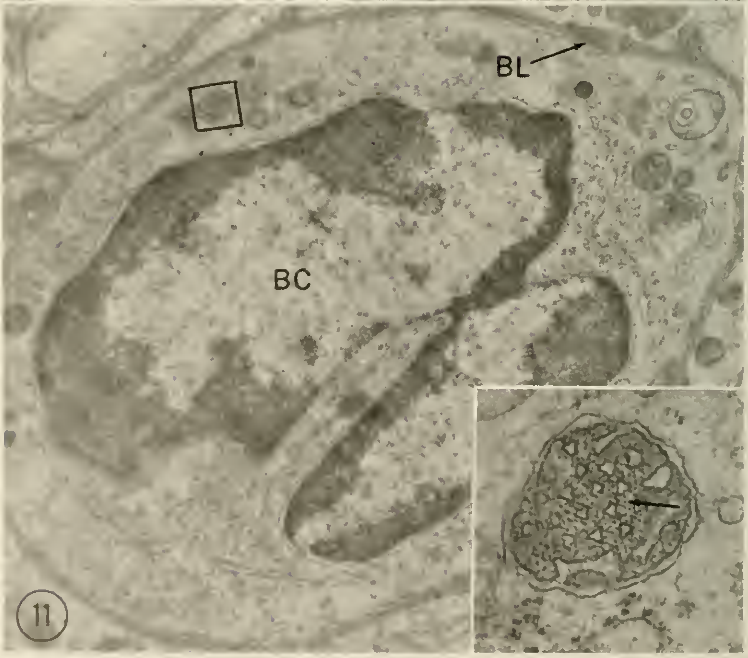
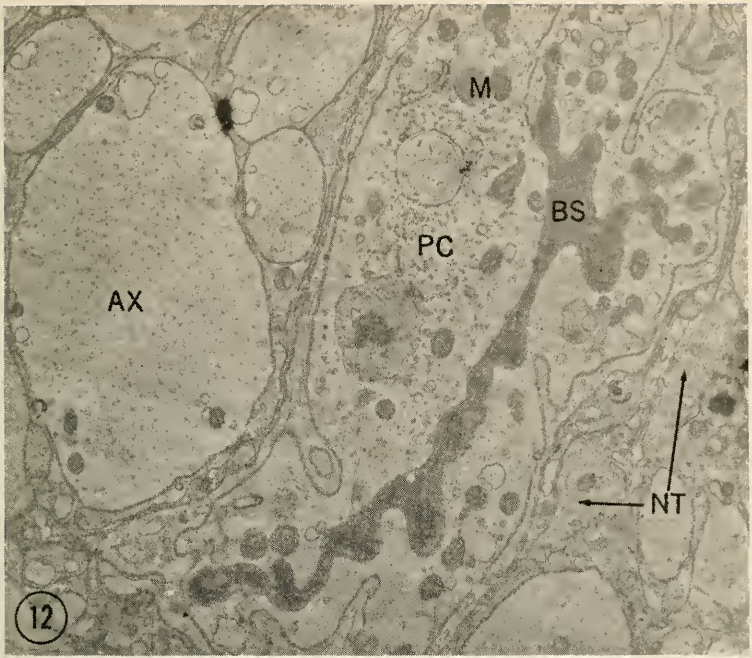


Fig. 10. Medulla internis. Blood sinus and neurons associated with an annulate axonal arrangement. Arrows indicate adhaerence plaques. LC-UA; ×4,795. Fig. 11. Medulla internis. Blood cell within a blood sinus. Note the dense particles in the mitochondrial matrix (arrow, inset). LC; ×14,286. Inset; ×60,480.



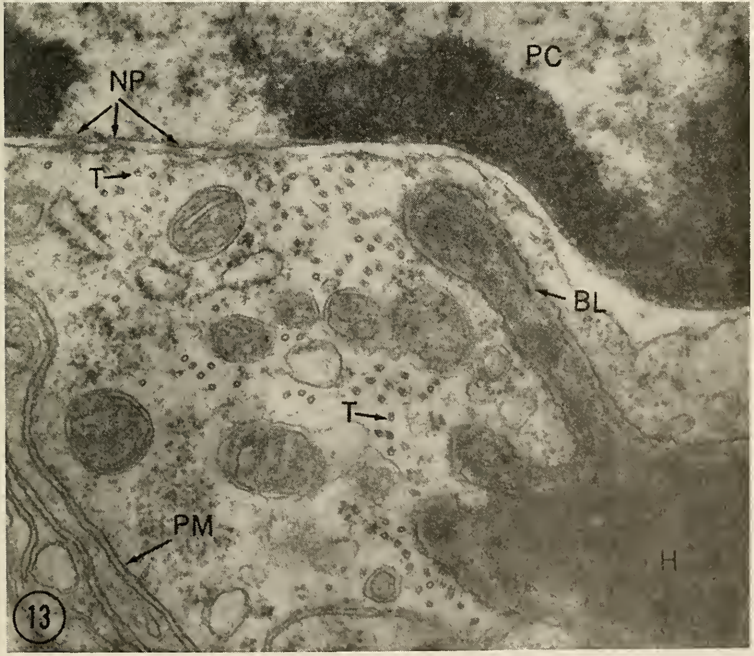


Fig. 12. Medulla internis. Blood sinus located in the ganglionic neuropil. The sinus appears collapsed in this section. LC; ×11,570. Fig. 13. Blood sinus and its supporting cell. Note the numerous microtubules seen in cross-section within the supporting cell cytoplasm. LC-UA; ×41,388.

mechanical damage to the tissue during preparative procedures. Granule release from axons has not been observed in the ganglionic neuropil.

Synaptic Areas. Synaptic fields are ubiquitous throughout the neuropil of the four optic ganglia. Two types of synapses can be distinguished on the basis of synaptic bouton contents: (1) boutons containing only clear vesicles 300-550 A in diameter (Figs. 7, 9), and (2) boutons containing both the clear vesicles and small, dense granules 600-1000 A in diameter (Figs. 30, inset; 32). Figure 30 and its inset show a large granulated axon synapsing with what appear to be several dendrites. It should be noted that clear vesicles and small secretory granules are present within the same axon and are clearly associated with the synaptic junction (Fig. 30 and inset.) At high magnification (Fig. 30 and inset), the electron-dense, membrane-limited granules are seen on both pre- and postsynaptic sides of the synaptic cleft, which is 225-250 A across. Separating the synaptic cleft into two equal halves is a thin, electron-dense band measuring about 30-40 A wide. Although clear vesicles appear to be associated with both pre- and postsynaptic membranes, the suggested direction of impulse conduction is shown by the arrow in Fig. 30 (inset). This conclusion is based on the preponderance of synaptic vesicles within one of the two synaptic terminals and the accumulation of dense material beneath the membrane of only one of the terminals. The arrow in the inset of Fig. 30 is also directed toward a clear vesicle which appears to be fused with the presynaptic membrane—vesicular structures also appear to be fused or closely associated with the postsynaptic membrane. Figure 32 shows synaptic junctions between a granulated axon and three dendrites, some of which are also granulated.

Synapses containing only clear vesicles are common within the ganglionic neuropil. Such regions are seen in Fig. 7 (arrows) and accumulations of clear vesicles at the presynaptic membranes and axoplasm is a common occurrence. Axons filled with dense granules are present near these synapses but apparently are not directly associated with them. Figure 9 shows an area of the medulla internis in which there are many synaptic junctions (arrows). Although many synapses are present, there is a noticeable absence of granulated axons. These synapses are not simple mono-terminal synapses but appear to be multiple synapses with one axon synapsing with several dendrites. The only synapses seen in this study were between neuronal processes and no axo-somatic synapses were observed. Large secretion granules (1000-1700 A in dia.) were never seen in synaptic boutons.

Glia-Neuron Relationships. Glial cells are indistinguishable from neurons when seen with the light microscope, but with the electron microscope some distinction can be made between them. The nuclei of glial cells are elongate and usually polymorphic, with masses of dense chromatin material near the nuclear envelope (Fig. 50). The cytoplasm of these glial cells extends as very

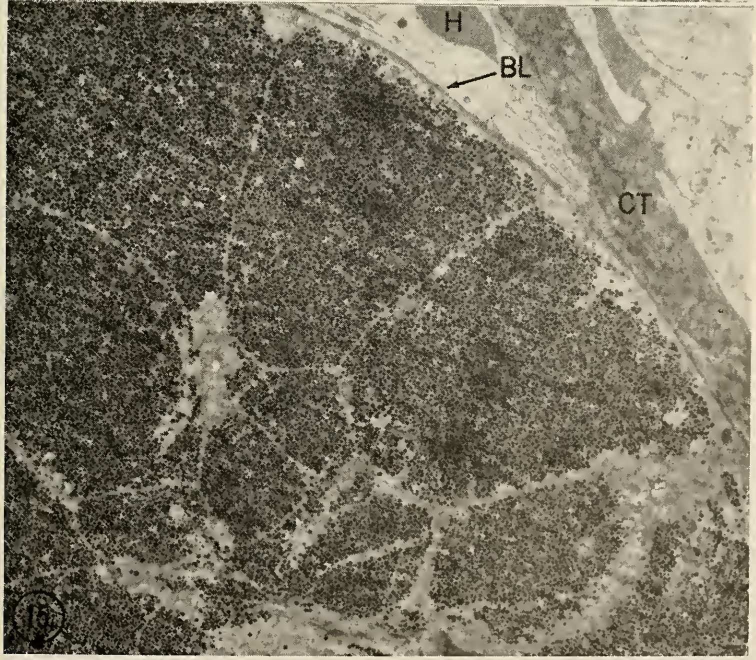


FIG. 14. Light micrograph of the internal optic chiasma. Ramon y Cajal silver-pyridine, ×648. FIG. 15. Low magnification micrograph of the sinus gland. Note that the granulated axon endings are encapsulated by an external sheath (BL). Karnovsky fixation, LC-UA; ×3,704.

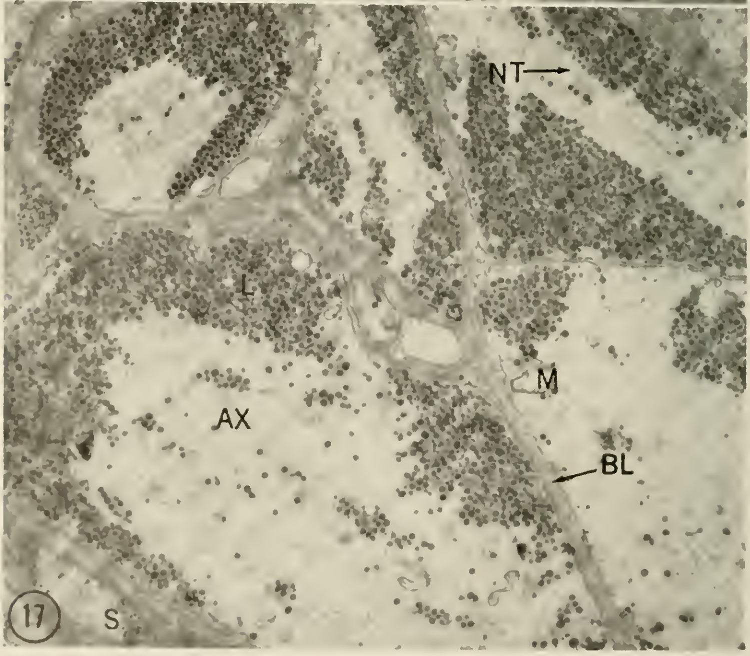


FIG. 16. Low magnification micrograph of the sinus gland. The gland appears lobulated in this section. Karnovsky fixation. LC-UA; ×4,760. FIG. 17. Sinus gland. Axons containing granules believed to be "en route" to endings in the sinus gland proper. Karnovsky fixation. LC; ×5,864.

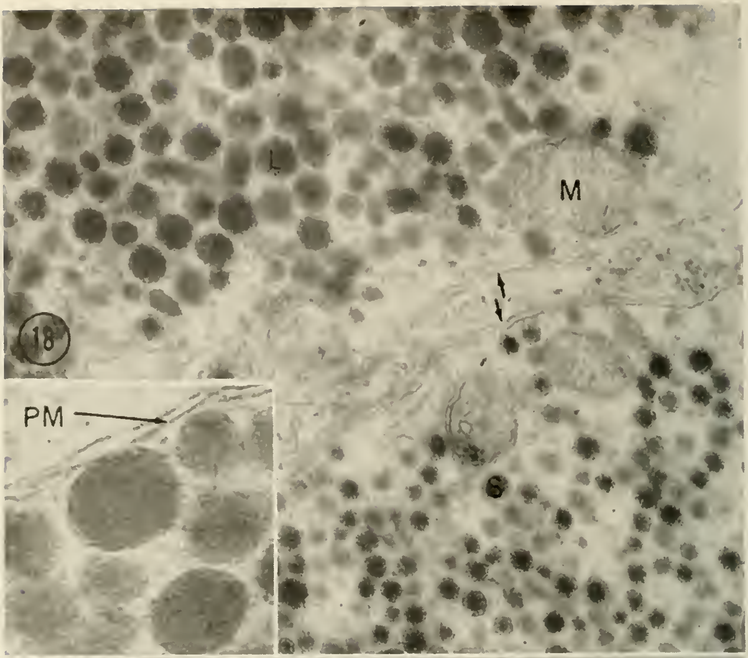
thin processes around axons of the neuropil, therefore very little cytoplasm is seen near the nuclei of glial cells (Fig. 50). Partial isolation of axons is achieved by virtue of their being surrounded by thin layers of glial cytoplasm. The axoplasm of adjacent axons is apparently continuous in some areas where axons are not separated by glial cytoplasm (large arrow, Fig. 20). Areas of continuity between axoplasm and glial cytoplasm can be seen in Figure 20 (small arrows) and in the inset.

Large nerve tracts exhibit a more complex form of glial insulation of their components than is seen within the ganglionic neuropil. The glial cell apparently produces a dense, amorphous "external lamina" which is deposited on the outer surface of the glial plasma membrane. Such laminae are present in the optic nerve (Fig. 50), and around large axons passing to the sinus gland (Fig. 17). Such an external lamina is never seen in association with glial cells of the ganglionic neuropil.

An unusual type of encapsulation of neuronal perikarya by glial cell processes can be noted in the X-organ of the optic tract (Figs. 25, 34, 35, 36). Cells designated as type 1 neurosecretory cells, which comprise the major cell type of the X-organ, are surrounded by a multi-lamellate sheath composed of numerous cytoplasmic processes (Fig. 25, large arrows). The multilamellate sheath is believed to be made up of very thin cytoplasmic processes of glial cells. This peculiar structural arrangement is unique to type 1 neurosecretory cells and is present in no other area of the eyestalk.

A final glial-neuronal association can be seen in the sinus gland (Figs. 15, 16). Here, glial cell processes encapsulate both the large groups of axons which end in the sinus gland, and also the individual axons which comprise these groups. In most cases, an external lamina is present on the outer surface of the glial plasmalemma (Figs. 15, 16, 17). Such a relationship would serve to insulate the axonal endings of the sinus gland.

Sinus Gland. The neurohaemal organ of the crayfish eyestalk is known as the sinus gland. This structure is located in an area lateral to the internal optic chiasma and corresponds to area 5 of Figure 1A. Electron microscopic examinations of this area show it to be composed of granulated axonal endings surrounded by blood sinuses and lacunae. Low magnification electron micrographs show the overall fine structure of the sinus gland in Figs. 15 and 16. Bundles of axons filled with secretion products are encapsulated by an external lamina, presumably laid down by glial cells (Fig. 15). Individual axons are also ensheathed by glial cytoplasm and compartmentalization of single axons gives this portion of the sinus gland a "lobulated" appearance. In the upper right corner of Fig. 15, portions of haemolymph can be seen as well as a large band of fibrillar connective tissue. Haemolymph seen here is probably contained in lacunar systems rather than in sinusoids having a "lining."



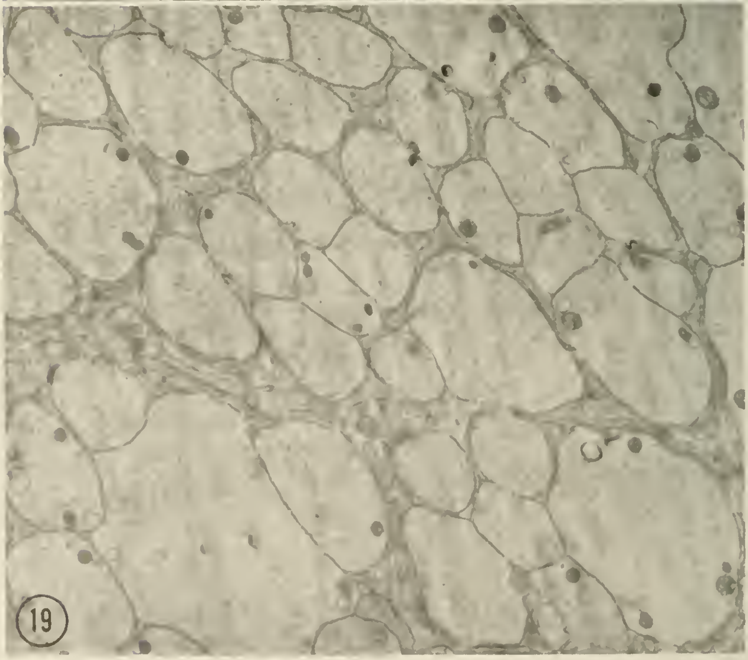
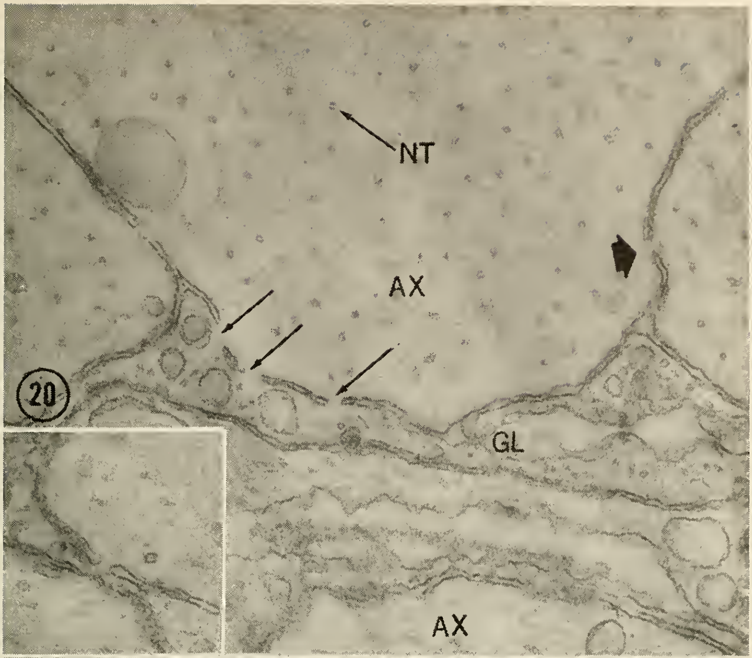


FIG. 18. Sinus gland. Note the presence of two granule types and also the suggestion of fusion of the granule membrane with the plasma membrane (inset). The two axon endings are separated by a glial cytoplasmic process (opposing arrows). LC-UA; ×42,536. Inset; ×95,238.
FIG. 19. Medulla externis. Cross-section of axonal processes. LC; ×7,807.



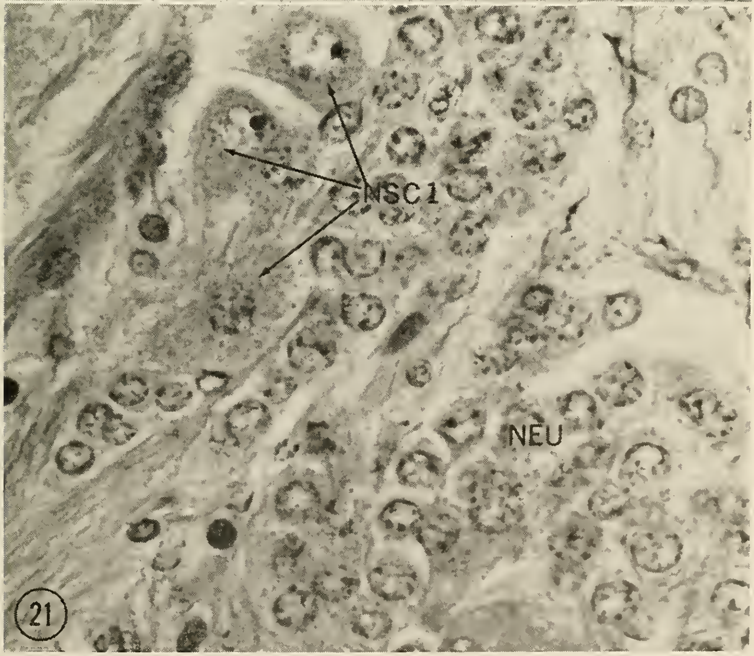


Fig. 20. Medulla externis. High magnification of a portion of Figure 19. Note the numerous neurotubules (NT) and areas of continuity between axoplasm and glial cytoplasm (small arrows and inset) and between adjacent axons (large arrow). LC; ×34,698. Inset; same. Fig. 21. Light micrograph of the X-organ. Note the large type 1 neurosecretory cells (NSC (Fig. 1). Iron-alum hematoxylin. ×648.

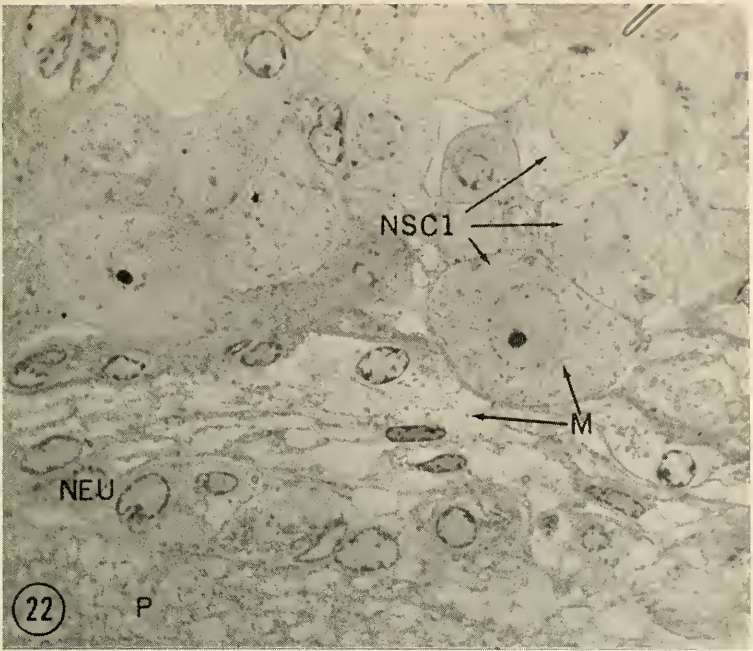
Figure 16 shows the lobulation of the sinus gland. Two bundles of granulated axons are separated by a clear space. Each of the two axonal bundles assumes the appearance of a lobule that is encapsulated by glial processes and their external laminae. A glial cell and processes of other glial cells are seen in Fig. 16, and apparently they contribute to the capsule ensheathing the lobules. The lower right portion of Fig. 16 shows large accumulations of dense material believed to be haemolymph.

Most of the granules seen in the sinus gland are 1000-1700 A in diameter, even though axons containing smaller granules (600-1000 A in diameter) are present (Fig. 18). Both granule types are membrane-limited, although in the smaller granules the space between their limiting membranes and contained granules is greater than with the large granules (Figs. 18, 37, 38, 39, 41). Small granules are ubiquitous within axons of the ganglionic neuropil (Figs. 6, 7, 24), but they are seldom seen within the sinus gland. The large granules are seldom seen within the ganglionic neuropil (Figs. 6, 7, 24), but make up almost the entire population of granules in the sinus gland.

Widespread evidence of granule release is absent in the sinus gland, but some suggestion of release can be seen in Fig. 18 and its inset. In the region of the arrows and in the inset, granule membranes appear to be in contact with the axolemma, which may represent a stage in the discharge of granules. Glial cytoplasm can be seen intervening between the two granulated axons (opposing arrows, Fig. 18).

Axons proximal to the sinus gland proper contain granules that appear to have been "en route" to axonal endings located near blood sinuses (Fig. 17). These axons show a reduced granule content and the granules are usually located just beneath the axolemma (Fig. 17). Axons leading to the sinus gland are extensively insulated by glial cells. A single axon located in the lower left corner of Figure 17 contains small neurosecretory granules (S).

X-organ. Associated with the medulla terminalis is a large group of cells collectively known as the X-organ. These cells are believed to produce neurosecretory materials which are transported along to the sinus gland. Electron micrographs of X-organ neurosecretory cells (type 1 neurosecretory cells) correspond to area 8 of Fig. 1A. The light microscopy of these cells has been described above and type 1 neurosecretory cells can be seen in Figs. 21, 22, and 23. General survey electron micrographs of these cells are seen in Figs. 34, 35, and 36. Type 1 neurosecretory cells apparently exhibit a cyclical activity in the production of their secretory material. Figure 34 shows a type 1 neurosecretory cell in what is suggested to be an inactive phase. Numerous Golgi bodies are present which exhibit a remarkable morphological uniformity in their configuration (inset). Small vesicular structures are seen near the Golgi bodies but no dense secretion granules are present, either associated with the Golgi bodies or free in the cytoplasm. Rough



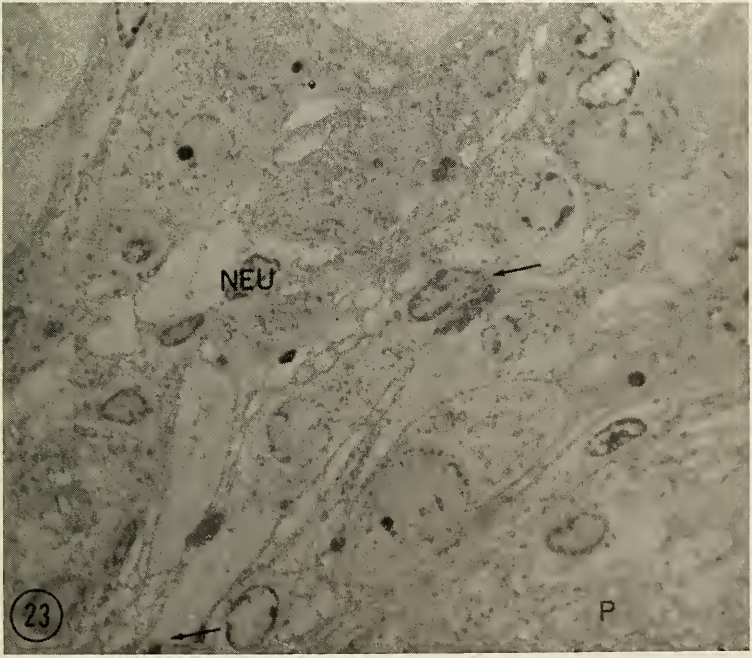


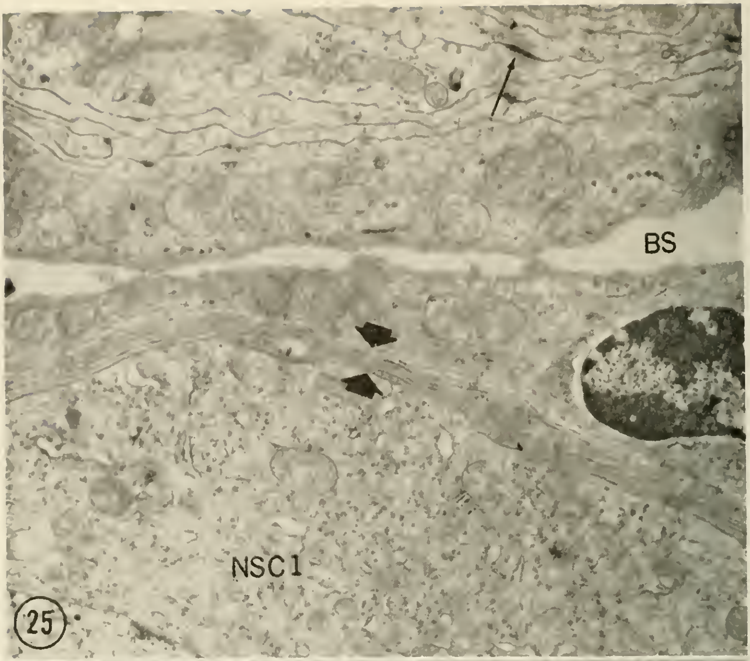
FIG. 22. Light micrograph of cells of the X-organ. Note the abrupt change of tissue into neuropil at the lower margins of the figure. Epon section stained with azure II and methylene blue. ×912. FIG. 23. Light micrograph of the X-organ. Note the extension of the cytoplasm (area between arrows) of the type 1 neurosecretory cell. Processes of these cells appear to be passing into the ganglionic neuropil. Numerous mitochondria can also be seen (M). Epon section stained with azure II and methylene blue. ×912.

FIG. 24. Medulla terminalis. General survey of the ganglionic neuropil containing granulated axons. Note the blood sinus (BS) in the lower left corner of the figure. Glut.—PO_4—OsO_4, LC-UA; ×17,280.

endoplasmic reticulum is abundant throughout the cytoplasm and occurs in vesicular form rather than as extensive lamellae. Free ribosomes are clustered in the cytoplasm as polyribosomes. The round nucleus is large (about 15 μ in dia.) and devoid of prominent masses of dense chromatin material. Many nuclear pores can be seen in the nuclear envelope. Prominent nucleoli are present, and usually two are seen in each nucleus even though more may be present and just not seen in random sections. Mitochondria are round in outline and are not abundant. Also, this cell type is surrounded by a multilamellate sheath which has previously been described.

Figure 35 shows a type 1 neurosecretory cell in what is suggested to be a regressive stage in its activity. A portion of the multilamellar sheath which surrounds type 1 neurosecretory cells is seen between the arrows in Fig. 35. Rough endoplasmic reticulum is quite similar to that found in what has been described as the inactive stage of this cell. Golgi bodies are not associated with dense granules; however, located near the Golgi bodies are peculiar structures which are believed to be multivesicular bodies. These bodies are surrounded by numerous "satellite" granules 600-1000 A in diameter (Figs. 35, 40, 43). It is suggested that the multivesicular bodies and their "satellite" granules are a morphological feature associated with the removal of secretion products, and possibly the secretory apparatus, from the cytoplasm of what has been described as the regressive stage of this cell.

The proposed mechanism by which these multivesicular bodies are formed will now be described, and individual events can be correlated with the series of electron micrographs in Fig. 43. By a "budding" process, the Golgi bodies appear to give rise to clear vesicles or slightly electron-dense granules (Fig. 43). As these bodies are derived from the Golgi elements, it is suggested that they aggregate and then merge (Fig. 43, A, B, C). Following the formation of a core structure for the multivesicular body, granules released from the Golgi apparatus probably become oriented around the periphery of the multivesicular body core as "satellite" granules (Fig. 43, D, E). The "satellites" then might pass from their peripheral location to the interior of the multivesicular body by fusion of their limiting membrane with the membrane surrounding the multivesicular body (Fig. 43, D, E). Granules which are assumed to have passed into the interior of the multivesicular body can be seen intact within this body (Fig. 43, D, and lower right micrograph). The membranous portions of granules within the multivesicular body, as well as the granule contents, are assumed to be eventually decomposed resulting in a structure which may be considered a mature multivesicular body or dense body (Fig. 43, F). The lower left micrograph of Fig. 43 is a relatively low magnification view of several multivesicular bodies and their satellite granules. The lower right micrograph of Fig. 43 is a high magnification view of what is suggested to be a "forming" multivesicular body. Mem-



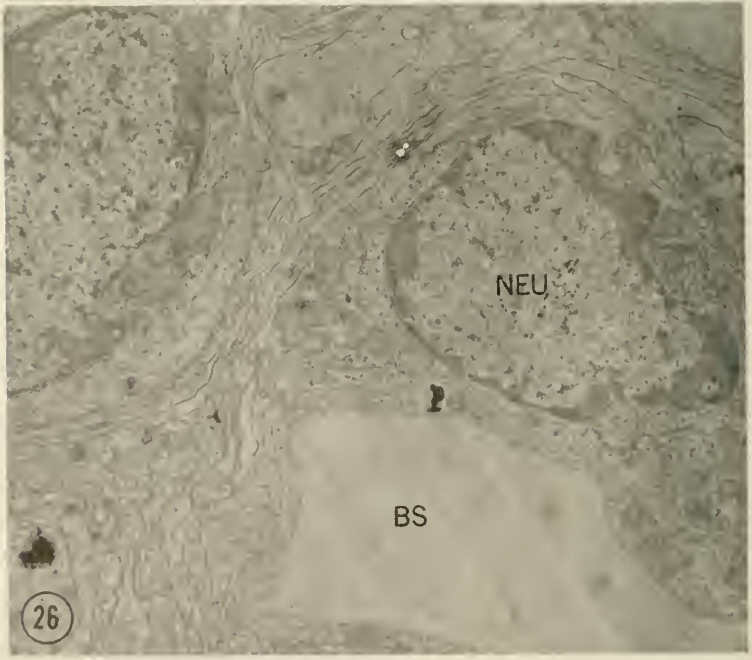


Fig. 25. Medulla terminalis. Blood sinus located near the X-organ. The type 1 neurosecretory cell is in what is considered to be an inactive stage. Note the multilamellate sheath (large arrows) surrounding this cell type. The small arrow indicates a possible adhaerence plaque. Glut.—PO_4—OsO_4, LC-UA; ×11,750. Fig. 26. Medulla terminalis. Neuron cell bodies located near a blood sinus in the ganglionic neuropil. Glut.—PO_4—OsO_4, LC; ×5,906.

FIG. 27. Medulla terminalis. High magnification micrograph of the perikaryon of a neuron located in the ganglionic neuropil. Note the numerous neurotubules and also the two adhaerence plaques. Glut.—PO_4—OsO_4, LC-UA; ×39,420.

branous portions of incorporated granules can be seen within this structure (arrow), and close observation of the limiting membrane of the multivesicular body suggests the passage of granules into its interior. These multivesicular bodies are conspicuously absent in the cytoplasm of type 1 neurosecretory cells which are massively granulated (Fig. 36).

Figure 36 shows the typical appearance of what is considered to be the active secretory phase of the type 1 neurosecretory cell. There seems to be more rough endoplasmic reticulum in these cells than in cells considered to be in the regressive and inactive synthetic phases. The cytoplasm is filled with large, electron-dense, membrane-limited granules. Numerous nuclear pores are present in the nuclear envelope and, although other cells of the eyestalk possess nuclear pores, the type 1 neurosecretory cells have many more of them as compared to other cell types of the eyestalk.

The inset (A) of Fig. 36 shows a Golgi apparatus of the type usually found in the active stage of this cell type. The short arrow indicates an electron-dense mass within a Golgi element and the long arrow indicates a stage in the probable "pinching off" of a membrane-limited, condensed granule from the end of a Golgi element. The membrane which surrounds the granule is apparently derived from the membrane of the Golgi element. Occasionally, small mitochondria can be seen in the cytoplasm. The multilamellate cytoplasmic sheath which insulates cells of this type is well demonstrated in Fig. 36 (large arrows).

Another feature of the type 1 neurosecretory cells believed to be actively engaged in synthesis is a structure termed an "onion body." The inset (B) of Fig. 36 shows such a body. These structures are multi-lamellate membrane systems arranged in whorls showing varying degrees of organization, and they frequently appear compact such as to resemble lysosomes. Onion bodies are apparently characteristic of the active phase of this cell type and are not often seen in other stages. Their function is not known.

In general appearance, Golgi bodies found in type 1 neurosecretory cells are quite similar to those of other neurons of the optic tract (Figs. 44-49). Portions of the concentric Golgi elements situated nearest the nucleus are vesicular and may be continuous with rough endoplasmic reticulum (arrow, Fig. 49). Granule release from the Golgi system appears to occur by their being "pinched off" the ends of the Golgi membranes (arrow, Fig. 45). Frequently, masses of electron-dense material are contained within the Golgi lamellae (arrows, Figs. 47, 48), and these are probably in a preliminary stage of condensation. The concentric lamellar arrangement of flattened Golgi elements is well demonstrated in Fig. 46.

Sinusoids. Blood sinuses are a common feature seen throughout the ganglionic neuropil (Figs. 2, 9, 10, 12). A blood sinus typical of the ganglionic neuropil is seen in Fig. 12. This sinus is collapsed and is contained

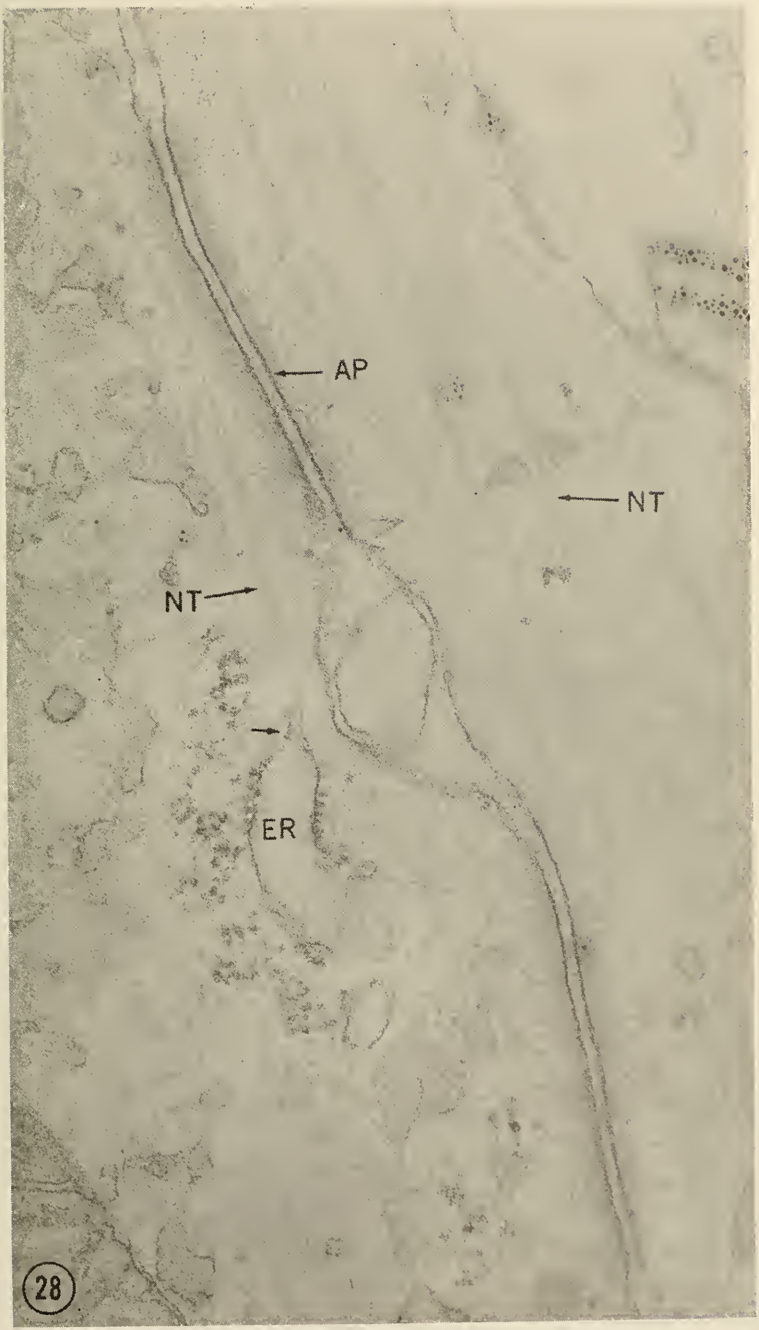
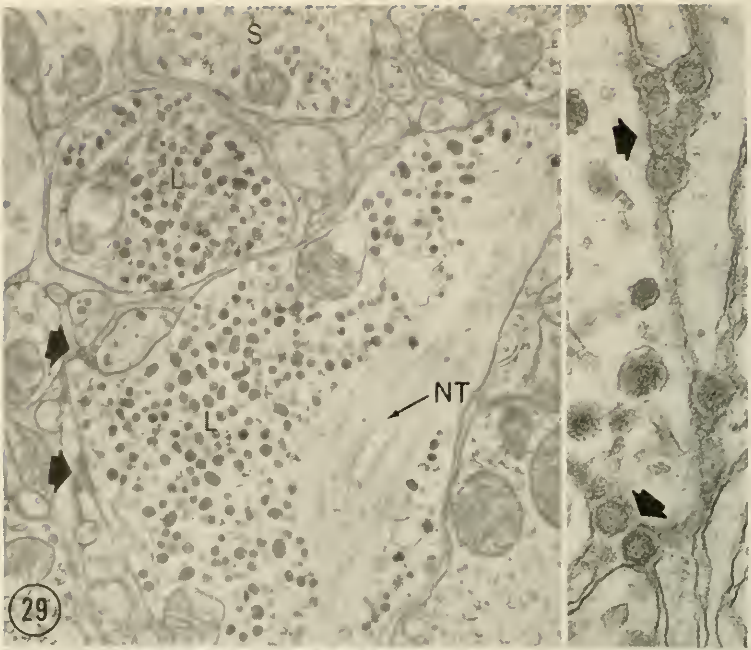


Fig. 28. Medulla terminalis. Two adhaerence plaques between adjacent neurons. Note the apparent continuity between the endoplasmic reticulum and a neurotubule (arrow). Glut.—PO_4—OsO_4, LC; ×63,072.



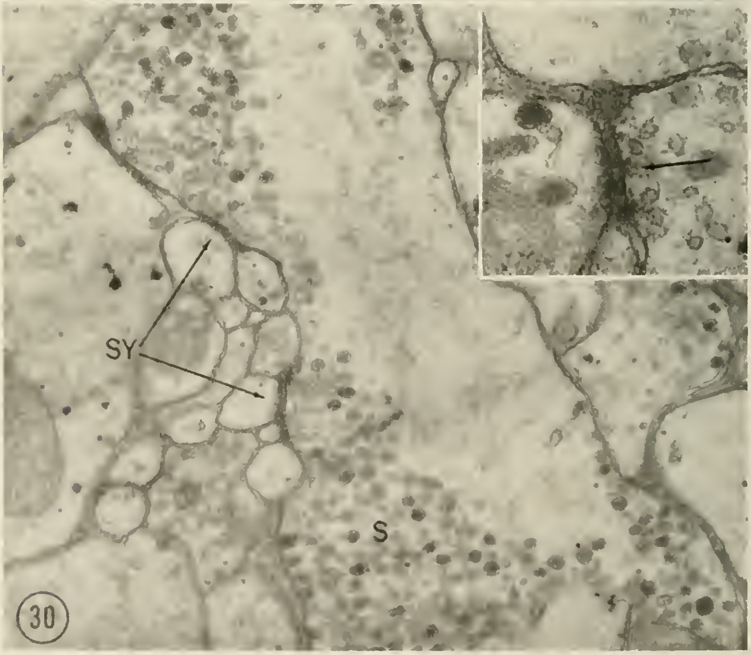
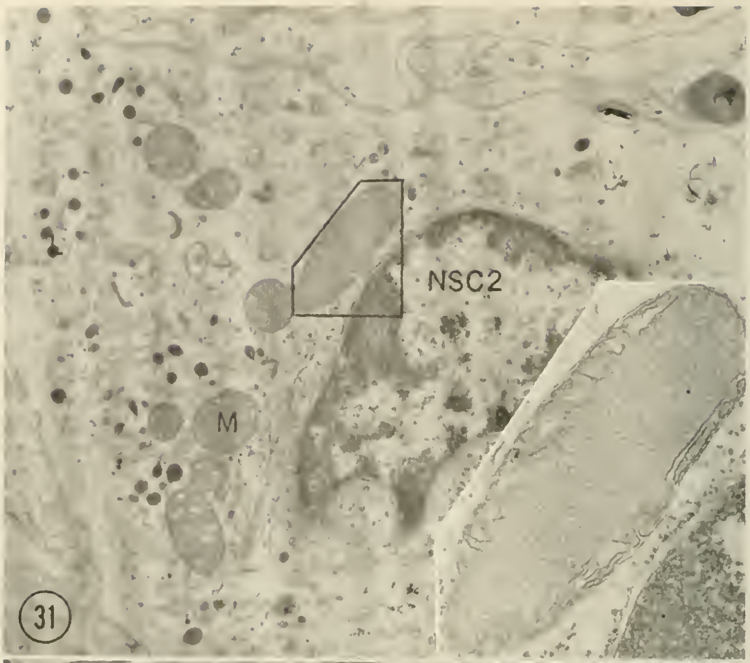


Fig. 29. Medulla terminalis. Area of granulated axons. Note the granules which appear to be outside the axons (large arrows and inset). Glut.—PO_4—OsO_4, LC-UA; ×28,154. Inset; ×63,492. Fig. 30. Medulla terminalis. Synaptic field containing both clear vesicles and dense granules. Note the accumulation of clear vesicles on the presynaptic membrane (inset, arrow) and also the thin dense band which separates the synaptic cleft into two equal halves. Glut.—PO_4—OsO_4, LC; ×14,392. Inset; ×52,910.

within a "sinusoidal supporting cell." Blood sinuses are lined with an amorphous substance called an external lamina, which is believed to be produced by the sinusoidal supporting cell (Fig. 13). The relationship between the sinus and its supporting cell is seen in Figs. 12 and 13. The sinusoidal supporting cells surrounding and containing the blood sinuses do not possess any unusual cytoplasmic features which could be related to their function. They do represent a barrier between the blood sinus and the neuropil (Fig. 12). Characteristic of the supporting cell cytoplasm are numerous microtubules which are about 250 A in diameter (Fig. 13); these are oriented parallel to the long axis of the sinus (Fig. 24). Figure 13 shows a blood sinus filled with an electron-dense, flocculent material which may be condensed haemolymph.

Occasionally, blood cells are trapped in a sinus during fixation procedures. Figure 11 shows a sinus containing a blood cell which possesses a large, irregularly-shaped nucleus. Located around the nucleus are sections of rough endoplasmic reticulum as well as many clusters of free ribosomes. The blood cell causes distention of the blood sinus which is probably in a more collapsed state in the living animal (Figs. 9, 10, 12). These blood cells contain dense secretion granules occasionally appearing crystalline and measuring 3000-7000 A in diameter. In some mitochondria of this cell type, the cristae appear triangular in cross-section with the equilateral sides about 420 A long. Scattered in the matrix of the prismatic cristae are small electron-dense particles about 90 A in diameter (Fig. 11, arrow, inset).

Optic Nerve. The medulla terminalis tapers into a structure known as the optic nerve, which provides a connection between it and the brain. Electron micrographs of the optic nerve correspond to area 9 of Fig. 1A. The optic nerve is composed of parallel bundles of axons passing between the brain and medulla terminalis (Fig. 50). Axon diameters are 0.1 μ to 18 μ. Associational areas are absent, with glial cells and connective tissue cells providing the only visible nuclei in the optic nerve (Fig. 50). Cytoplasmic extensions of glial cells provide a means whereby axon bundles and individual axons are insulated or separated from one another. Glial cytoplasm surrounds numerous axons and apparently the cytoplasm of each glial cell is distributed over a very large area, since few glial nuclei are present within the optic nerve (Fig. 1). Very little perinuclear cytoplasm is associated with the glial nuclei (Fig. 50). Occasional areas of fibrous connective tissue provide major divisional partitions between large tracts in the optic nerve (Fig. 50). Numerous mitochondria are seen beneath the axolemmae of axons within the optic nerve and large axons filled with small dense secretion granules are occasionally seen. Such axons are seen infrequently and the location of their perikarya is unknown.



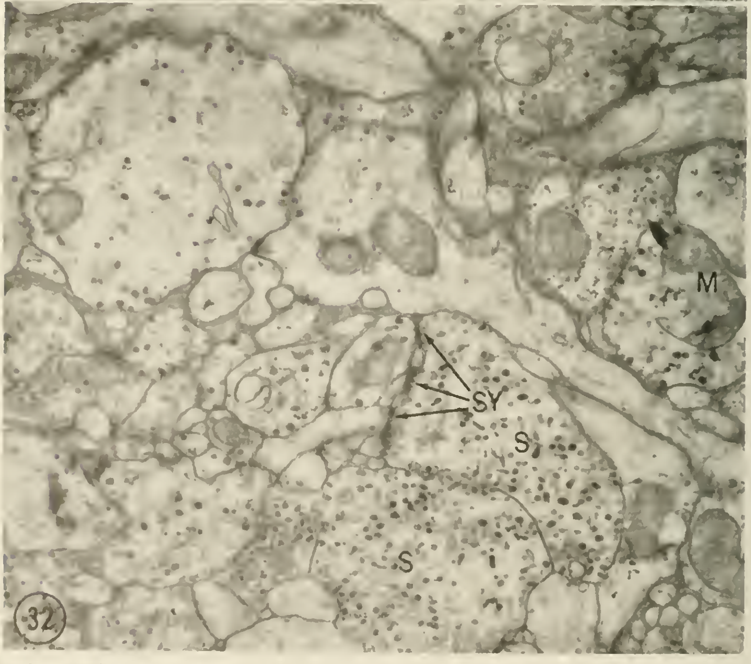


FIG. 31. Medulla terminalis. Type 2 neurosecretory cell. Inset shows a "crystalline" mitochondrion. Glut.—PO_4—OsO_4, LC; ×11,750 Inset; ×34,560. FIG. 32. Medulla terminalis. Granulated axons within an area containing a tri-synaptic axon ending. Note the presence of both clear vesicles and dense granules in the presynaptic axoplasm. Glut.—PO_4—OsO_4, LC-UA; ×14,391.

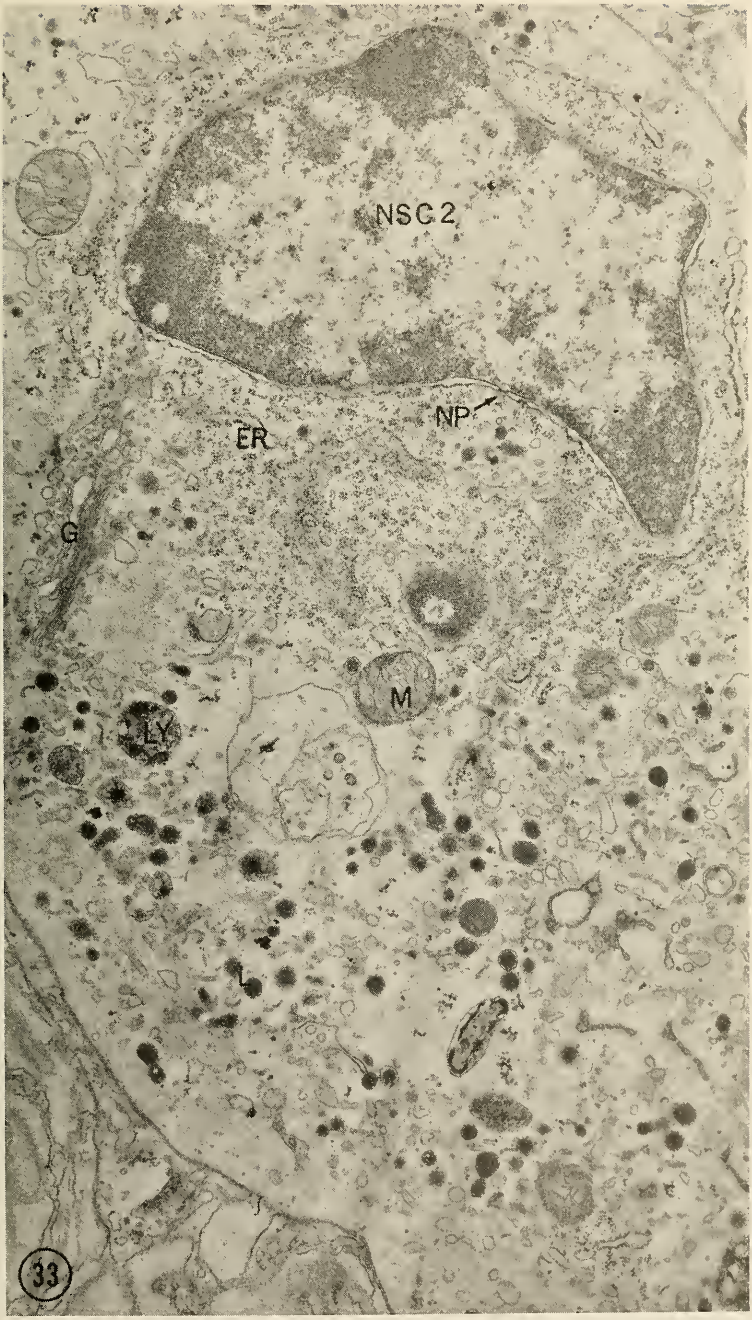


FIG. 33. Type 2 neurosecretory cell of the medulla terminalis. Note the accumulation of endoplasmic reticulum around the nucleus. Secretory granules appear to be associated with the endoplasmic reticulum and with the Golgi apparatus. Glut.—PO_4—OsO_4, LC; ×18,144.

DISCUSSION

Ganglia of the crayfish eyestalk are masses of neuropil surrounded in part by layers of neurons which serve to interconnect each of the four ganglia. Such an innervation would provide for coordination, not only within a single ganglion, but also between different ganglia, both eyestalks, and the brain as well. The reader is referred to the work of Seabrook and Nesbitt (1966) for a detailed description of the innervation of crayfish optic ganglia and the innervations of neurons which are located in the optic chiasmata and on peripheral margins of the ganglionic neuropil. The largest ganglion, the medulla terminalis, receives fibers from the other three ganglia and is connected to the deutocerebrum by the optic nerve. The medulla terminalis receives fibers from the opposite medulla terminalis, supraesophageal ganglion, and other lower centers as well as reciprocally innervating these structures. Seabrook and Nesbitt (1966) postulate that the medulla terminalis serves as an associational area for optic and olfactory stimuli.

Axons of retinular cells in the ommatidia pass posteriorly through the basement membrane into the lamina ganglionaris which appears to serve as an associational area to modify or coordinate sensory impulses initiated in the retinular cells. Such associational phenomena are believed to occur throughout the optic tract of crayfish. Clusters of neurons near the lamina ganglionaris surround the retinular axon bundles as they pass into the lamina ganglionaris. The area below the basement membrane is highly vascularized and may be related to neurohormonal control of retinal pigment activity. Located in this area are proximal pigment granules, retinular axons, and neuron cell bodies, but few neurosecretory axons are present. It is assumed that neurohormonal elements controlling pigment migration arise in ganglia further down the optic tract and are transported to the pigmented cells by the vascular system—this assumption would explain the extensive vascularization of the zona fasciculata.

The numerous synaptic fields in the neuropil of the four optic ganglia suggest that the optic ganglia are mainly involved in associational activities. Two types of synaptic endings are seen with the electron microscope: (1) synaptic endings containing both clear vesicles, 300-550 A in diameter, and membrane-limited granules measuring 600-1000 A in diameter, and (2) synaptic endings containing only the clear vesicles. In the neuropil of the optic ganglia, axons filled with large neurosecretory granules (1000-1700 A in diameter) are very seldom seen.

The small secretion granules seen throughout the neuropil of the crayfish optic ganglia closely resemble granules observed in nervous systems of other organisms, such as the leech (Hagadorn *et al*., 1963), granules in beta nerve fibers (Knowles, 1965), type 2 granules of the aphid corpora cardiaca (Bow-

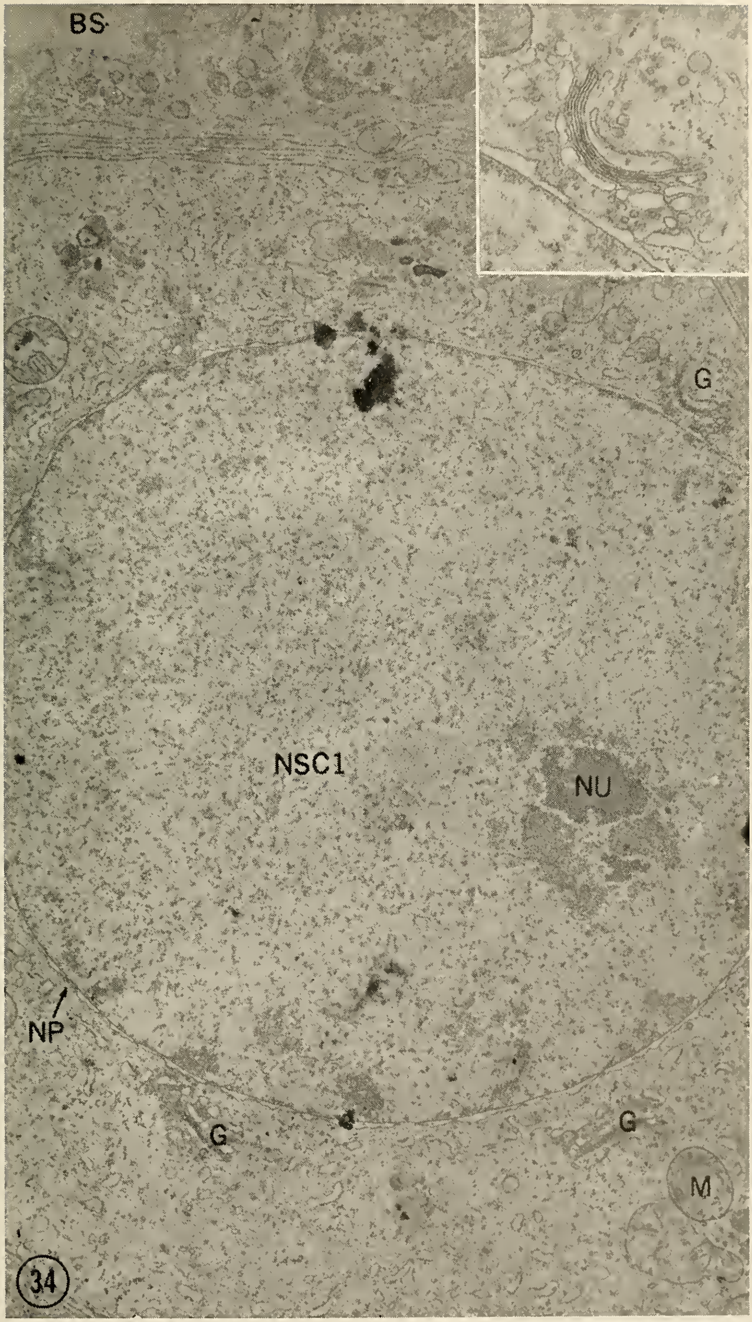


Fig. 34. Type 1 neurosecretory cell of the X-organ. This cell is considered to be in an inactive secretory phase; note the numerous Golgi bodies (inset). Glut.—PO_4—OsO_4, LC; ×9,120 Inset; ×21,715.

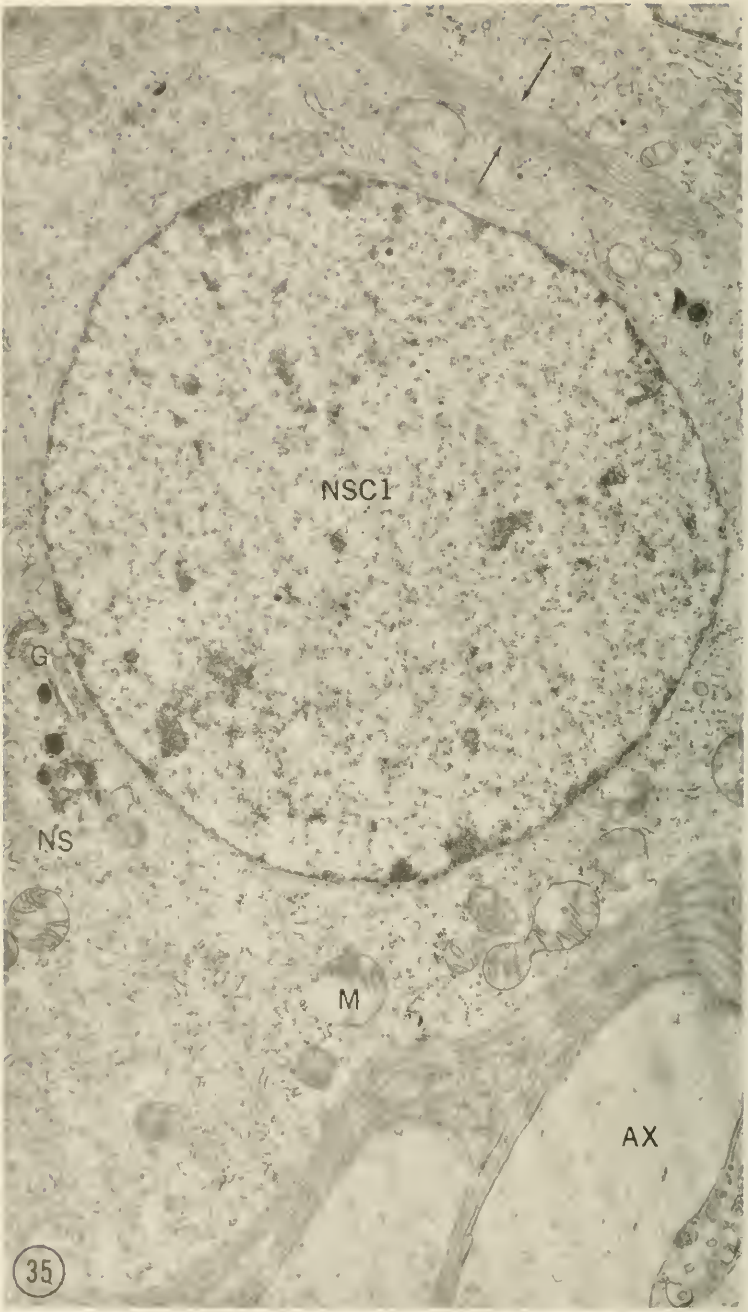


FIG. 35. Type 1 neurosecretory cell of the X-organ. This cell is considered to be in a regressive phase of secretory activity. Note the multivesicular bodies with their "satellite" granules and also the multi-lamellate insulation around the cell (small arrows). Glut.—PO_4—OsO_4, LC; ×9,120.

ers and Johnson, 1966), insect corpora cardiacum granules (Scharrer, 1963), granules of the planarian nervous system (Morita and Best, 1966), and granules of the dwarf crayfish central nervous system (Fingerman and Aoto, 1959). Hagadorn *et al.* (1963) suggest that these granules contain an adrenergic instead of a cholinergic compound and that such granules are "normally" found in the nervous tissue of such organisms. Knowles (1965) suggests that granules found in a general class of fibers, termed beta fibers by him, probably contain a nonproteinaceous substance. Recently, fluorescence techniques have shown that there are adrenergic neurons in the central nervous system of Crustacea (Elofssen *et al.*, 1966). Dopamine was demonstrated as the dominant catecholamine present, even though smaller amounts of 5-hydroxytryptamine and 5-hydrohytryptophan were also demonstrated. Elofssen *et al.* (1966) postulate the presence of two types of monoamine terminals: (1) those located in associational areas such as the medulla terminalis, medulla externis, protocerebrum, and ventral nerve cord; and (2) monoamine fibers exhibiting no fluorescence and located in areas receiving impulses from sensory structures such as the lamina ganglionaris. The above data support the hypothesis that the small granules (600-1000 A in dia.) are adrenergic and contain monoamines.

It is quite apparent that in the crayfish optic neuropil, small granules are a common component of a class of synaptic endings which also contain clear vesicles (synaptic vesicles). The neuronal perikarya which produce these granules were not observed, which suggests that the formation or at least the condensation of these granules may occur at a site other than the perikaryon. Many neuron cell bodies were observed in this study and in no case were dense secretion granules seen within the perikarya, although dense secretion granules were seen in neuronal processes of the neuropil. This observation supports the assumption that granule condensation occurs at a site other than the neuron perikaryon. Knowles (1965) proposes that these granules contain amines and possess a crystalline core. In the present study, the small granules (600-1000 A in dia.) exhibit a particulate, electron-dense core, and only the large granules (1000-1700 A in dia.) occasionally exhibit a crystalline core. More often, the core of the large granules appears particulate rather than crystalline.

Knowles (1965) suggests that granules of the general size class of less than 1000 A in diameter are associated with neural activities requiring short-term control, and granules measuring 1000-2000 A in diameter are associated with long-term activities requiring sustained periods of hormone release. This hypothesis is tenable on a morphological basis since in the present study neurosecretory granules 600-1000 A in diameter were seen only in synaptic endings of nerve processes and were never seen in large numbers near a vascular supply; e.g., as groups of axon endings near a blood sinus. However,

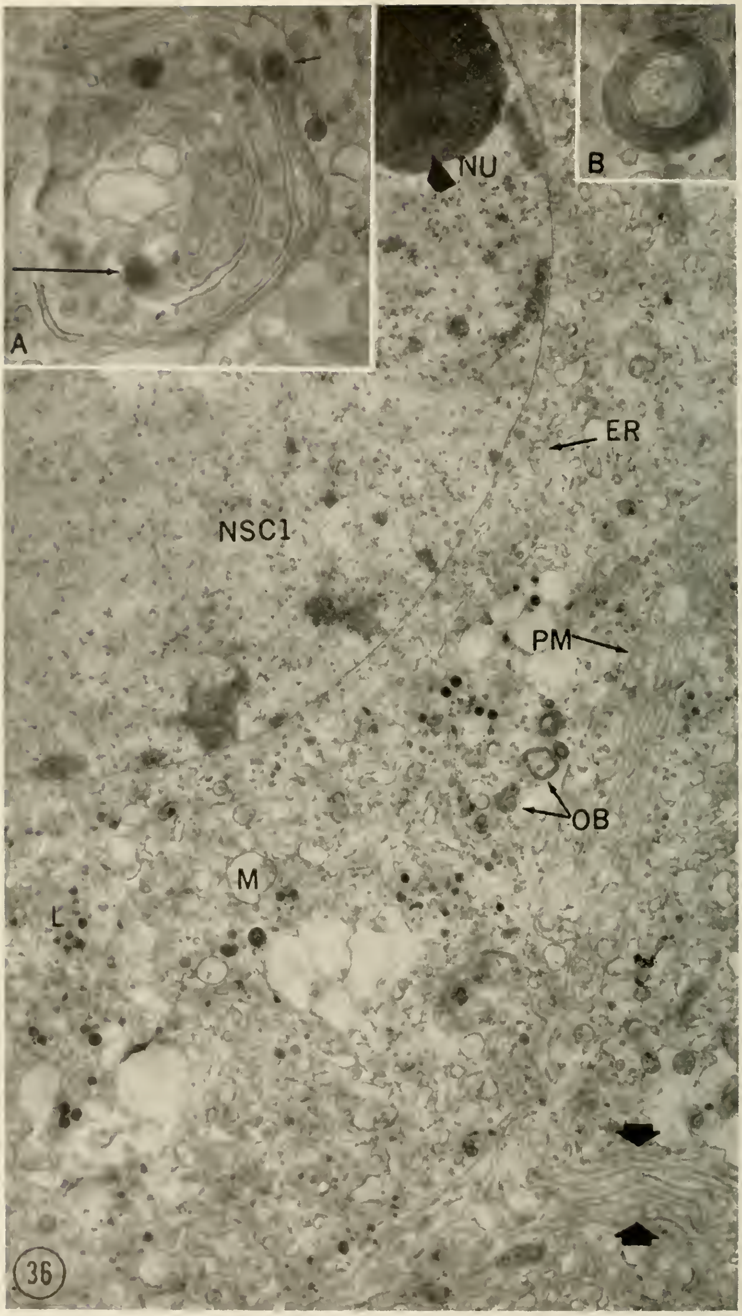
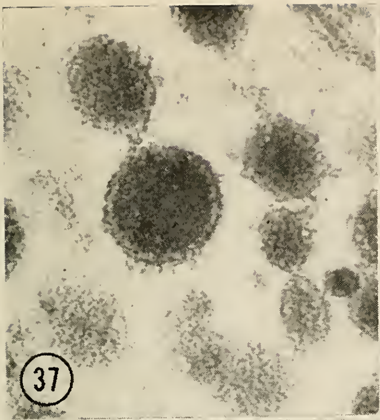
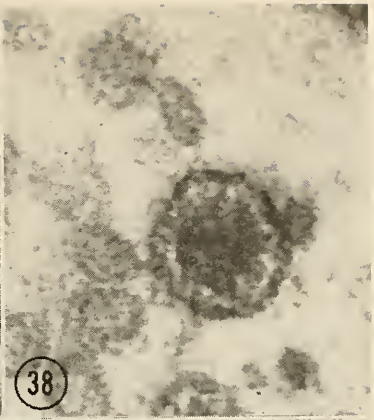
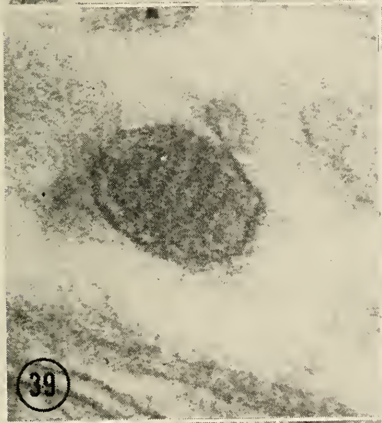
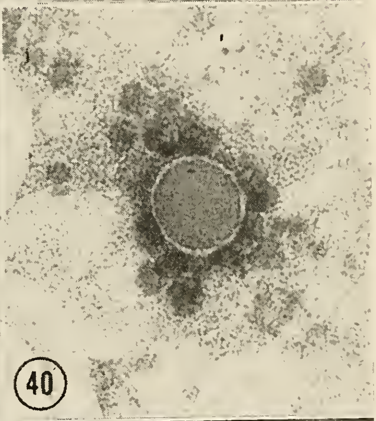


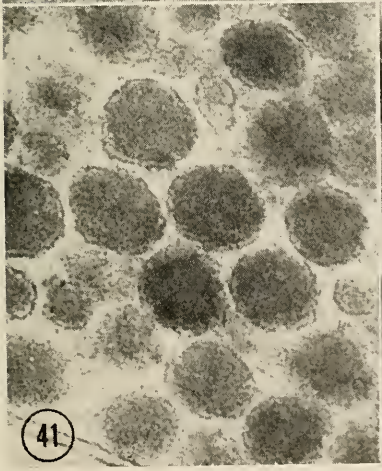
Fig. 36. Type 1 neurosecretory cell of the X-organ. This cell is considered to be in an active phase of secretory activity. Note the presence of onion bodies in the cytoplasm and in the inset (B). Large arrows delimit the typical multi-lamellate sheath around this cell type. Inset A shows secretion granules (arrows) within Golgi membranes. Glut.—PO_4—OsO_4, LC; ×7,934. Inset A; ×31,746. Inset B; ×28,571.











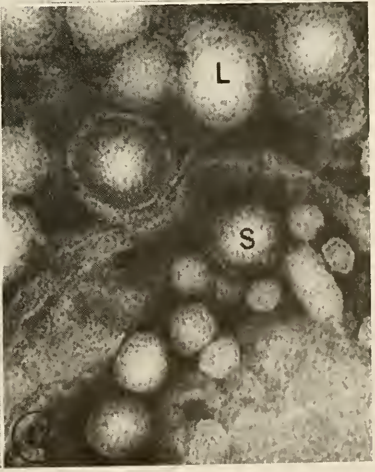


Fig. 37. Large neurosecretory granule. Glut.—PO_4—OsO_4, LC-UA; ×124,164. Fig. 38. Small neurosecretory granule. Glut.—PO_4—OsO_4, LC-UA; ×170,064. Fig. 39. Large "crystalline" neurosecretory granule. Glut.—PO_4—OsO_4, LC-UA; ×170,064. Fig. 40. Multivesicular body with "satellite" granules. Phosphotungstate negative staining. ×80,417. Fig. 41. Large neurosecretory granules from the sinus gland. Karnovsky fixation. LC-UA; ×95,238. Fig. 42. Large and small neurosecretory granules. Phosphotungstate negative staining. ×80,417.

large neurosecretory granules (1000-1700 A in dia.) comprise the bulk of the granules of the neurohaemal sinus gland and are infrequently seen in random sections of ganglionic neuropil. Since the functions of the sinus gland include control of ovarian size, molt inhibition, and a hyperglycemic factor (Kleinholz, 1966), such responses could be considered maintenance phenomena requiring a sustained release of secretion product, whereas small neurosecretory granules associated with synapses are involved with the transmission of nerve impulses and their effects are short-lived.

Various workers have suggested that the clear vesicles (300-550 A in dia.), located in synaptic boutons also containing dense granules (600-1000 A in dia.), may be membranous remnants of granules which were previously released into the synaptic cleft or bouton axoplasm (Hagadorn *et al.*, 1963; Holmes and Knowles, 1960, Gershenfield *et al.*, 1960; de Robertis, 1964). The possibility exists that the two vesicle types represent two chemical compounds, with one facilitating the release of the other. That is, the clear vesicle could contain acetylcholine and this transmitter could be required to effect the release of the contents of the electron-dense granule (Hagadorn *et al.*, 1963; Fridberg, 1963; Rinne and Arstila, 1966; Werman, 1966). Landolt and Sandri (1966), in studies of synapses in wood ant nervous tissue, showed that in some cases cholinergic synapses are present in boutons possessing both synaptic vesicles and dense-centered vesicles measuring 700-1000 A in diameter. Evidently, the true significance of the two vesicle types of the synaptic boutons must await more intensive studies.

Evidence obtained in this study suggests that synaptic transmission is effected in a classical way by the release of a transmitter substance into the synaptic cleft (de Robertis, 1964). Clear vesicles were sometimes seen to be fused with the presynaptic membrane, which suggests that they were in the process of releasing their contents into the synaptic cleft. Small vesicles of unknown significance were also seen to be associated with the postsynaptic membrane. This association was observed in planarian nervous tissue by Morita and Best (1966). A dense band was noted in some of the synaptic clefts of synapses of the crayfish eyestalk. Such a band would appear to divide the cleft into two equal halves, and, although its function is unknown, similar structures were reported by Normann (1965) in the nervous system of *Calliphora erythrocephala.*

Intercellular junctions resembling synaptic complexes were noted between the axolemmae of adjacent axons and occasionally between axons and the plasma membrane of nerve cell bodies. The opposing junctional membranes have an increased electron-density although no apparent increase in thickness can be ascertained. The cytoplasm beneath the junctional membrane is dense, although no apparent substructure is seen. Some of these specialized regions resemble adhaerence plaques, which were described in the leech nervous

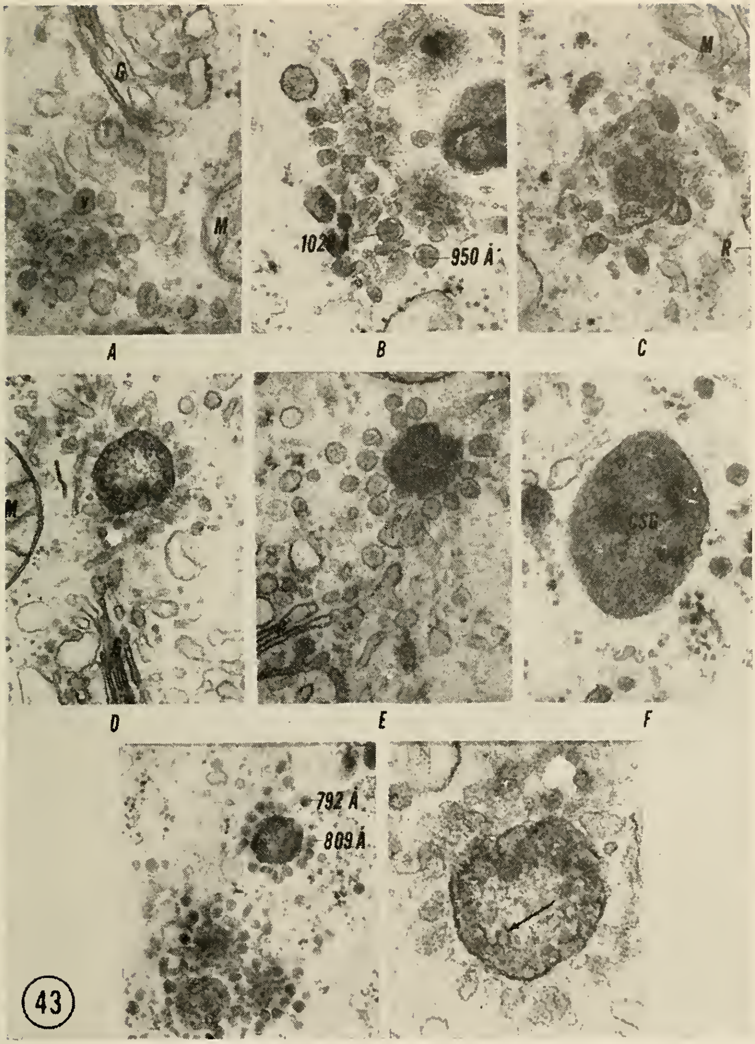


Fig. 43. Suggested stages in the formation of multivesicular bodies which are seen in what has been described as the regressive secretory phase of type 1 neurosecretory cells. See the text for a discussion of the steps in the formation of these structures. Glut.—PO_4—O_sO_4, LC; A, B, C, E, F, ×43,154; D, ×32,850. Lower left; ×22,046. Lower right; ×65,700.

system (Coggeshall, 1965). These membrane complexes are assumed to provide structural support of the nervous tissue by interconnecting various neuronal processes and perikarya.

Neuronal cell bodies located peripherally around the ganglia and also neurons found within the ganglionic neuropil, exhibit no evidence of neurosecretory activity. "Neurosecretion" as used here, refers to the process whereby neurons produce a substance which is packaged into electron-dense granules and transported along axons to a location some distance from the neuronal perikaryon, where the substance is liberated. Two classes of neurosecretory neurons can be postulated: (1) neurons which produce a condensed secretion product which passes along axons to an area near a vascular supply where it is released into the vascular system, and (2) neurons which synthesize a product which passes along axons to an ending called a synaptic bouton where it is released as a transmitter substance. Products of class 1 neurosecretory neurons are known as neurohormones and products of class 2 neurosecretory neurons are known as neurotransmitters or neurohumors. This distinction is not exactly in keeping with the original definition of "neurosecretion" based on the affinity of some neurons for "neurosecretory stains" as postulated by Bargmann and Scharrer (1951), but it is somewhat in keeping with the discussion of Knowles (1965) concerning this matter. Bern (1963) suggested that the "neurosecretory stains" of light microscopy may not always be used with confidence in distinguishing between neurosecretory and non-neurosecretory neurons.

Common to neurons associated with the optic tract are numerous neurotubules 200-250 A in diameter. Tubules seen within the perikarya are not oriented in any particular direction, but those found in neuronal processes are oriented parallel to the long axis of the process and occasionally exhibit lengthy longtiudinal profiles. In one case, continuity was observed between a neurotubule and endoplasmic reticulum. Although such association of "microtubular" structures with other cytoplasmic organelles has been postulated and occasionally reported (Slautterback, 1963; Sandborn, 1966), the occurrence of such relationships is very seldom seen. Such an association suggests that such neurotubules might serve as intracellular transport systems (Slautterback, 1963; Sandborn, 1966). In this case, ions or small molecules might be transported to or from the cisternae of endoplasmic reticulum.

Glial cells are most easily recognized by an elongate or irregularly-shaped nucleus and very little perinuclear cytoplasm. Plasma membranes of glial cells often show a dense surface layer, or external lamina, which is especially characteristic of glial cells that insulate axons of large nerve bundles, such as those of the optic nerve and sinus gland tracts. Insulating glial cells of the ganglionic neuropil, however, do not usually possess an external lamina. Cytoplasmic continuity between axons or axons and glia is occasionally seen. Landolt and Ris (1966) observed similar structures in insect neural tissue and postulated a dynamic relationship which would include the regulation of interneuronal transfer of information. Such structures were not often observed in the present study since axons within the ganglionic neuropil are usually found to be insulated by a sheath of glial cytoplasm.

Since crustaceans have an "open" circulatory system, blood sinuses and lacunae comprise portions of this system. Sinuses of the neuropil are limited by supporting cells. Lining the sinus is a layer of electron-dense material which probably is produced by the supporting cells. Numerous microtubules are seen within the cytoplasm of supporting cells and appear to run parallel to the long axis of the sinus. Burton (1966a, 1966b) suggested that some microtubules may serve as cytoplasmic supportive elements; perhaps microtubules of supporting cells function in this manner. Within the neuropil, sinuses are occasionally seen to contain blood cells. Occasionally, mitochondria of these blood cells exhibit triangular cristae when seen in cross-section. Associated with these tubular cristae are numerous electron-dense particles which are scattered in the mitochondrial matrix. Similar mitochondria were seen in alpha cells of the lizard pancreas (Burton and Vensel, 1966), and in other cell types as well (Gray, 1960; Ashhurst, 1965). The electron-dense particles, about 90 A in diameter, may correspond to the "elementary particles" described by Fernandez-Moran *et al.* (1964).

With the light microscope, certain cells of the medulla terminalis are indistinguishable from neurons and glial cells, but with the elecron microscope these are obviously neurosecretory elements. Although not seen with great frequency, these cells show structural evidence of much secretory activity, with the secretion product being membrane-limited granules 1500-2100 A in diameter. These cells, designated as type 2 neurosecretory cells, apparently are few in number and the location of their axonal terminals is unknown. Further, granules of the size produced by this cell type have not been observed in axons within the neuropil, and no vascular supply has been seen associated with these cells.

It is difficult to ascribe a function to these cells, especially in the light of the multiple physiological responses elicited by extracts of the medulla terminalis (Kleinholz, 1966). In his studies of the neurohormonal activities of crab eyestalks, Enami (1951a) determined that extracts of the medulla terminalis and brain caused pigment concentration in black chromatophores and pigment dispersion in white chromatophores. In a later paper (1951b), he attempted to locate the source of this hormone in cells which he classified as gamma neurosecretory cells located in the brain and medulla terminalis. Although he incorrectly stated that the secretion was of nuclear origin, he did distinguish between these cells and cells which he called beta cells. Beta cells correspond to the giant neurons of the medulla terminalis X-organ which are classified as type 1 neurosecretory cells in the present paper.

Separation of eyestalk extracts have demonstrated the presence of a substance in the medulla terminalis which will cause pigment dispersion in brachyuran melanophores (Kleinholz, 1966). It is unknown whether the hormone discussed by Enami corresponds to that of Kleinholz, but perhaps

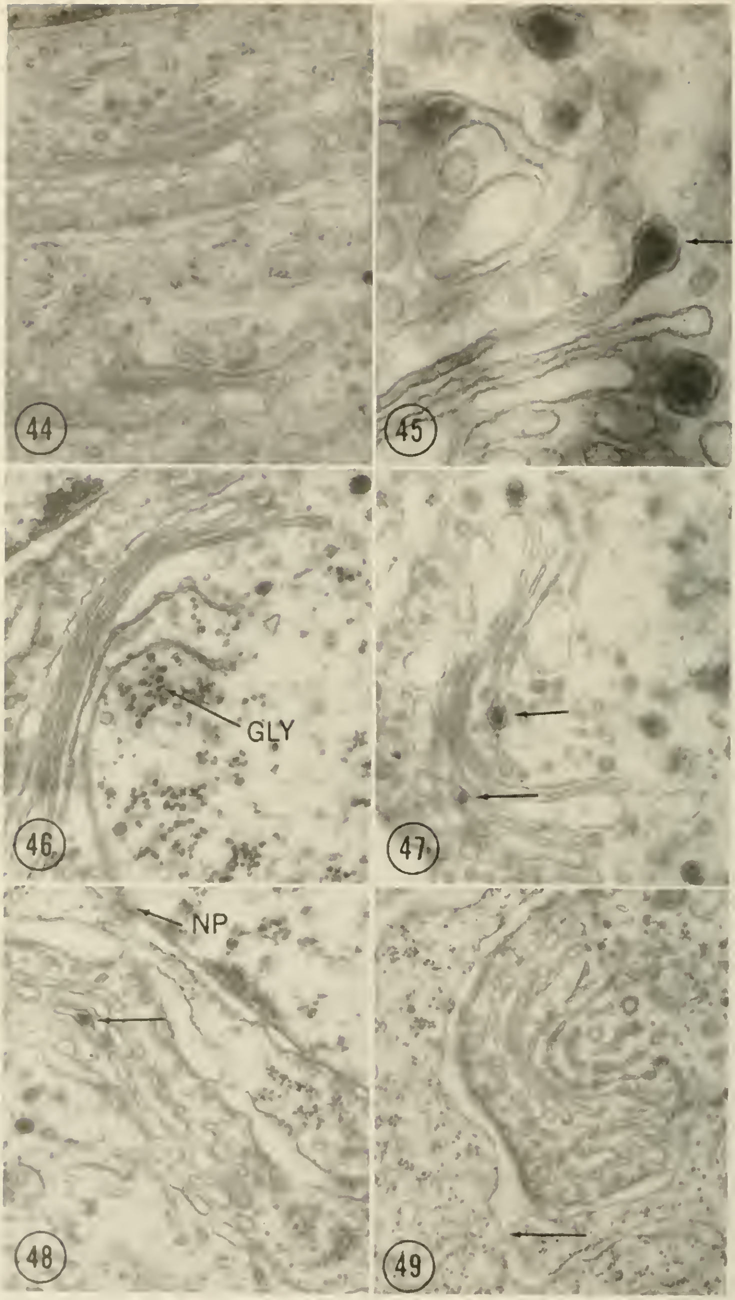


Fig. 44. Golgi apparatus from the inactive stage of type 1 neurosecretory cells. LC-UA; ×29,959. Fig. 45. Granules believed to be "budding" from the Golgi membranes (arrow). LC-UA; ×56,688. Fig. 46. Golgi apparatus with nearby glycogen granules. LC-UA; ×38,264. Fig. 47. Golgi apparatus containing secretion material believed to be in the process of condensation (arrows). LC-UA; ×39,319. Fig. 48. Golgi apparatus containing secretion material believed to be in the process of condensation (arrow). LC-UA; ×39,319. Fig. 49. Golgi ap-

the granules seen in type 2 neurosecretory cells in the present study may contain a hormone similar to that described by Enami and Kleinholz, and which is relatively specific in its action. This assumption is based on the fact that type 2 neurosecretory cells are not abundant; therefore one can assume that only small quantities of its hormone are produced, as would be expected for a hormone of limited action. The cells were not seen associated with a vascular supply. Seemingly, the specific-acting hormone described by Enami (1951a, 1951b) and Kleinholz (1966) is produced by type 2 neurosecretory cells, since these were the only neurosecretory cells observed in the neuropil of the medulla terminalis.

The sinus gland of the crayfish has been studied with the electron microscope by Fingerman and Aoto (1959), and that of the land crab by Hodge and Chapman (1958). The two granule sizes described were: (1) 500-1000 A and 1500-2000 A in a land crab (Hodge and Chapman, 1958), and (2) 500-1000 A and 1000-1600 A in a crayfish (Fingerman and Aoto, 1959). These measurements are in accordance with those made in the present study (600-1000 A and 1000-1700 A). The great majority of the granules seen in the sinus gland are large (1000-1700 A in dia.). It is difficult to believe that the major granule type found in the sinus gland is responsible for all the physiological activities attributed to this gland, since small granules, although not abundant, are nevertheless present. Some morphological evidence does exist for the release of two granule types from the sinus gland. Both large and small neurosecretory granules can be seen in contact with the plasma membrane of their respective axons, presumably fixed while in the process of release.

The X-organ has been shown to be the source of compounds found in the sinus gland (Passano, 1953; Welsh, 1941; Bliss *et al.*, 1954; Messner, 1966). Cyclical activity of secretory cells is well-known, although cyclic activity within the X-organ of crayfish has not been demonstrated (Pyle, 1943). Scharrer (1966) demonstrated cyclical activity in the prothoracic glands of cockroaches and correlated structural modifications with such activity. Morita and Best (1965) discussed cyclical activity in neurosecretory cells of planaria, and Pyle (1943) noted cyclical changes in the contents of the crayfish sinus gland during molting, but he was unable to relate these to the appearance of cells in the X-organ or to demonstrate any cyclical activity in X-organ cells. In the present study, structural evidence was presented that seems to indicate cyclic activity in cells of the X-organ. These cells, (type 1 neurosecretory cells) are extremely large and correspond to the giant beta cells described by Enami (1951b). They produce granules corresponding in

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paratus. Note the suggestion of continuity between the endoplasmic reticulum and the Golgi membranes (arrow). LC-UA; ×26,455. Note: All material shown in this Plate was fixed in phosphate-buffered glutaraldehyde and OsO_4.

size and morphology to the large granules of the sinus gland. Secretion products produced in this cell type appear to be synthesized in the endoplasmic reticulum and condensed into electron-dense, membrane-limited granules by the Golgi complex, a general sequence elucidated for pancreatic acinar cells by Caro and Palade (1964). Various investigators reported secretion products associated with the Golgi complex and have suggested that the function of the complex is to "package" such material (Hirsch, 1961; Scharrer and Brown, 1961, 1962; Bern *et al.*, 1961; Bern, 1963; Maillard, 1963; Scharrer, 1963; Morita and Best, 1965). The morphology of the Golgi elements seen within the type 1 neurosecretory cells of the X-organ, as well as the Golgi elements of the optic tract, exhibit morphological uniformity.

Figures 34, 35, and 36 illustrate the proposed stages in the cyclical morphology of the type 1 neurosecretory cells of the X-organ. The active stage of this cell contains numerous cytoplasmic granules apparently produced in the endoplasmic reticulum and condensed by the Golgi apparatus. Characteristic of the cytoplasm of this cell type is the presence of vesicular endoplasmic reticulum. Ribosomes are seen clustered in the cytoplasm and mitochondria are present although not numerous. Unique to the active stage of this cell are numerous, concentric whorls of membranes called onion bodies. The function of these structures is unknown but they may be lysosomes and represent a preliminary stage of autolytic removal of secretion products from the cytoplasm. Although the cell designated as active in the present study contains many cytoplasmic granules, the presence of onion bodies may reflect a preliminary regression in the synthetic activity of this cell. Scharrer (1964b, 1966) described structures similar to these onion bodies in regressing prothoracic glands of the cockroach and ascribed an autolytic function to them.

Figure 35 shows a regressive stage of the type 1 secretory cells. Endoplasmic reticulum is typically arranged and no secretion granules are present in the cytoplasm. The presence of large, membrane-limited bodies surrounded by a single layer of "satellite" granules is characteristic of this stage of activity. These structures may be associated with the removal of synthetic products released by the Golgi apparatus. Figure 43 illustrates the proposed morphological stages by which products of the Golgi apparatus are sequestered and condensed into a multivesicular body which is presumed to eventually form a dense body. The formation of multivesicular bodies in the removal of secretion products in cells of the anterior pituitary has recently been reported by Smith and Farquhar (1966). Such a mechanism provides for the removal from the cytoplasm of residual secretion products during the regression of secretory activity by this cell.

The inactive stage of this cell type contains no multivesicular bodies and no secretion granules, suggesting that the cell might be in a stage preparatory to resuming its secretory activity. The production of secretion granules by type 1 neurosecretory cells is not synchronous, thus it is possible to find cells representing all stages of secretory activity in the same X-organ. Further study is necessary to relate the activities of the sinus gland and X-organ to their physiological functions.

Perhaps a situation exists in which the smaller secretion granules are concerned with one general class of physiological responses, possibly involving pigment activity, whereas the large granules are concerned with a second general class of physiological responses such as those associated with molt inhibition, ovarian development, and hyperglycemia. The neurohormonal control of molt inhibition, ovary size, and hyperglycemia may be reciprocal or antagonistic depending up on differential thresholds of tissue sensitivity to neurohormonal blood levels. This assumption is a reasonable premise for attributing to one granule type the control of a general class of physiological responses. Since the large granules (1000-1700 A) are much more abundant in the sinus gland, it would not be unreasonable to suggest that they might be involved in a broader spectrum of physiological responses than the small granules (600-1000 A). Also, the abundance of large granules might reflect a need for sustained release of a secretory compound. However, the possibility exists that one granule type can possess several compounds with hormonal activity when broken down from the "parent" compound (Kleinholz, 1966; Schreiner, 1966).

The cyclical nature of molting and its chronological relation to gonadal development suggest a correlation between control of these processes and the cyclical activity of the sinus gland (Pyle, 1943), and X-organ. Further, evidence showing a cyclical change in the staining affinity of material in the sinus gland prior to molting (Pyle, 1943) can be correlated with the present study on the fine structure of the sinus gland. The major portion of the sinus gland is composed of large neurosecretory granules, and the presence of small neurosecretory granules is negligible. To obtain a change in the staining affinity of the sinus gland, it follows that the only substance within the gland which is present in quantities large enough to account for such chromatic differences at light microscopic levels must be the large neurosecretory granules.

Previously it was suggested that type 2 neurosecretory cells may have some control over pigment activity in chromatophores. Evidence of a second and smaller granule type (600-1000 A) within the sinus gland was also given. It is assumed that small neurosecretory granules of the sinus gland may play a role in the control of retinal pigment migration and also chromatophore activity (erythrophores and melanophores). Chromatographic separation of brachyuran eyestalk extracts has shown two major peaks, one of which represents the erythrophore concentrating hormone and the other showing

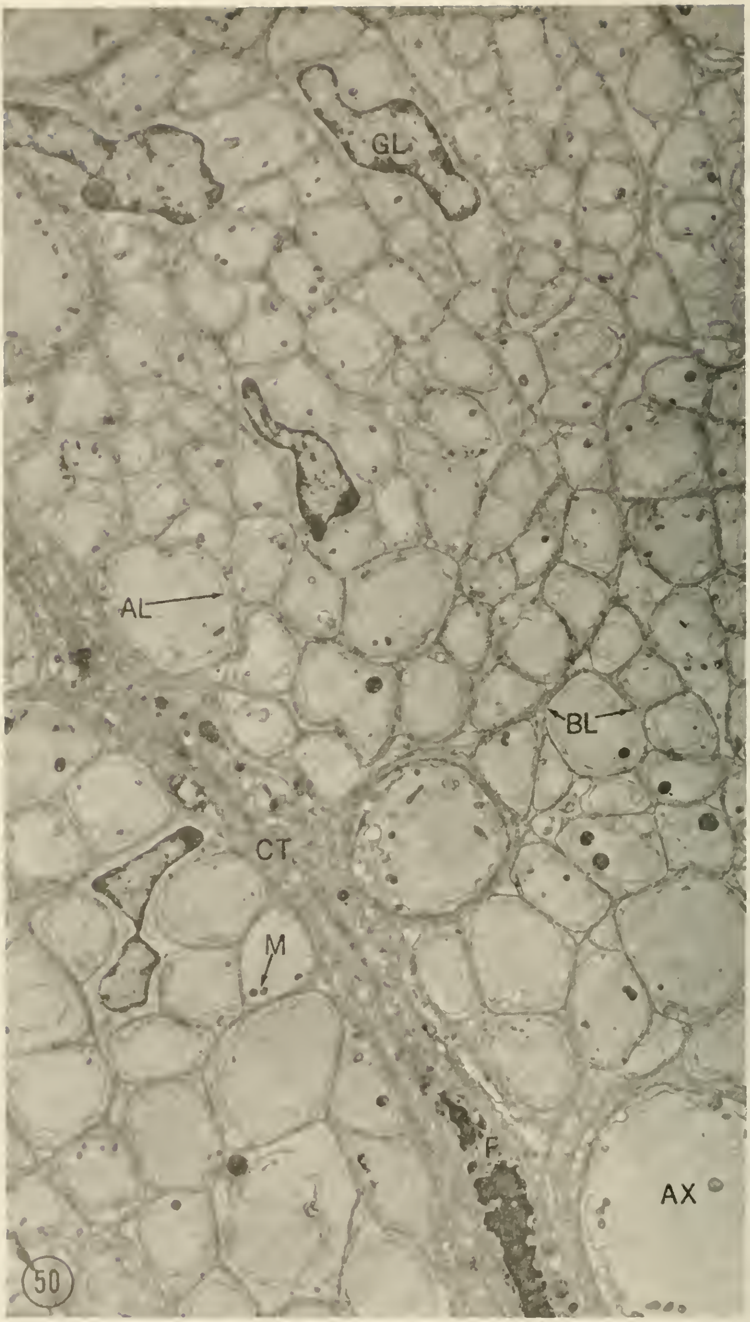

FIG. 50. Optic nerve cross-section. Note the nuclei of the glial sheath cells. Most of the axons contain mitochondria located just beneath the axolemma. Note the large mass of connective tissue which passes through the optic nerve in the lower left corner of this figure. LC-UA; ×2,258.

no chromatophoric activity (Kleinholz, 1966). The substance of a smaller peak near that of the erythrophore concentrating hormone effects both distal retinal pigment movement and brachyuran melanophore pigment dispersion. The major peak and its accompanying smaller peaks, which exhibit no major chromatophoric activity, could represent substances identified with the control of molt, ovarian size, and possibly hyperglycemia.

It is evident from electron microscopic observations that the X-organ is highly vascularized. Since several layers of glial cytoplasm separate type 1 neurosecretory cells of the X-organ from adjacent blood sinuses, it is doubtful that granules are released from axons in such regions. The type 1 neurosecretory cells are the only optic tract cells which exhibit such an extensive encapsulation by glial processes. Similar multi-lamellate glial encapsulations of nerves have been reported in the prawn (Heuser and Doggenweiler, 1966). These workers suggest that such nerves will conduct impulses at a more rapid rate than nerves lacking such a covering. It is proposed that this structural enhancement of type 1 cells in the crayfish optic tract may serve to compartmentalize the cells and facilitate rapid conduction. This specialization is undoubtedly significant since it is not seen elsewhere in the eyestalk.

Cells resembling type 1 neurosecretory cells have also been seen with the electron microscope in portions of the deutocerebrum and in ganglia of the ventral nerve cord, thus suggesting a widespread neurosecretory involvement in physiological activities. Extraction techniques applied to selected portions of the crustacean nervous system (medulla terminalis, sinus gland, brain, etc.) have demonstrated control of physiological processes by cells located elsewhere in the organism (Enami, 1951a, 1951b)..

The problem of release of secretory granules has been an intriguing one and has yet to be settled. Ultrastructural studies have provided little information on granule release, although several theories have been set forth. Some authors suggest that neurosecretory granules may become dissociated in the axoplasm such that constituent molecules can then diffuse through the cell membrane (Fridberg, 1963a, 1966; Hagadorn *et al*.. 1963). Some authors have observed points of apparent fusion between the granule-limiting membrane and the axolemma, which would allow the release of contained granules into the extracellular space (Bern, 1965; Weiss, 1965; Normann, 1966). Normann (1966) suggests that due to Brownian movement, granules are constantly moving and thus granules frequently make contact with the plasma membrane. The presence of an action potential at the membrane when the granules strike it would facilitate their release by permitting the granule membrane to interact with the axolemma. The necessary condition of having an action potential present to facilitate granule release presupposes that if no action potential were present, no granules would be released. Regulation of the amount of secretory material released would depend upon the length of duration of the action potential at the membrane, and continual release of the secretory product would be prevented in the absence of an action potential. Scharrer (1963) and Johnson (1966) have observed axon endings filled with granules and clear vesicles, the latter being typically associated with synaptic junctions. They suggest that the dense granules are decomposed into the smaller clear vesicles which then move to the cell membrane for release of their contents. The mechanism of release from the cell could be similar to that proposed by Normann (1966). The decomposition of the dense granules could involve the separation of the neurohormone from a carrier substance prior to release of the hormone (Schreiner, 1966). Observations made in the present study support the theory that granule release is effected by the fusion of the granule-limiting membrane with the axolemma.

SUMMARY

1. The optic ganglia of the crayfish *(Orconectes nais)* have been studied with the electron microscope. Light microscopy was correlated with the electron microscopy.

2. Four classes of neurons are distinguished on the basis of types of elementary granules produced by them:

   a) Type 1 neurosecretory cells contain electron-dense, membrane-limited granules measuring 1000-1700 A in diameter. These cells are located primarily in the X-organ.

   b) Type 2 neurosecretory neurons contain electron-dense, membrane-limited granules measuring 1500-2100 A in diameter. These cells appear to be located only in the medulla terminalis.

   c) Neurons, the processes of which contain dense, membrane-limited granules measuring 600-1000 A in diameter. Such granules are located in the sinus gland and in synaptic boutons. Boutons containing this granule type also contain clear vesicles 300-550 A in diameter.

   d) Neurons, the processes of which contain no electron-dense granules. The synaptic boutons of axons of these neurons contain only clear vesicles 300-550 A in diameter, which are assumed to be typical synaptic vesicles.

3. Only one granule type is seen in each axon or cell perikaryon, although clear vesicles may be present.

4. Golgi elements contain an electron-dense material which appears to become budded from the ends of the Golgi element as mature secretion granules. Clear vesicles also appear to become budded from the elements of the Golgi apparatus and their contents may condense later at another site in the axoplasm.

5. Ganglionic neuropil is vascularized with blood sinuses but no evidence of release of secretion products into them has been seen. The neurohaemal organ of the eyestalk is the sinus gland, which is described. The major granule type of the sinus gland measures 1000-1700 A in diameter and corresponds to granules produced by type 1 neurosecretory cells in the X-organ. A second granule type measuring 600-1000 A in diameter is occasionally seen in the sinus gland.

6. Four granule types can be identified in the optic ganglia of the crayfish:

a) Large neurosecretory granules which measure 1000-1700 A in diameter are the primary component of the sinus gland and X-ogan secretory cells (type 1 neurosecretory cells), and are occasionally seen in axons of the ganglionic neuropil.

b) Small neurosecretory granules measuring 600-1000 A in diameter are occasionally seen in the sinus gland. This granule type is ubiquitous within the axons and synaptic boutons of the ganglionic neuropil.

c) Clear vesicles (300-550 A in diameter), assumed to be synaptic vesicles, are seen in synaptic boutons of the ganglionic neuropil and also in terminals containing the small neurosecretory granules.

d) Granules which measure 1500-2100 A in diameter are seen only in the perikarya of type 2 neurosecretory cells of the medulla terminalis.

7. Axon bundles and individual axons, especially those of the optic nerve and sinus gland, are encapsulated by cytoplasmic processes of glial cells.

8. Large granules in the sinus gland are identical with those found in type 1 neurosecretory cells of the X-organ, and it is suggested that these granules are involved in the control of molt inhibition, ovary size, and hyperglycemia. It is also suggested that granules of type 2 neurosecretory cells, and the small granules seen in the sinus gland, are involved in the control of chromatophore and retinal pigment activity.

9. A cyclical sequence of secretory activity is proposed for the type 1 neurosecretory cells of the X-organ. Morphological features of such activity are described and the activity of these cells is related to sinus gland activity.

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COMPARISONS BETWEEN NEW METHODS FOR ANALYSIS OF IONOSPHERIC RECORDS

By

G. D. Shilling and H. Unz

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Comparisons Between New Methods For Analysis of Ionospheric Records*

G. D. Shilling[1] and H. Unz[2]

ABSTRACT

The uniform lamination analysis method is used for the solution of the simplified integral equation which relates the electron density profiles in the ionosphere with the average measured virtual heights, taking the wave refractive index as the kernel function. The arbitrary auxiliary function is chosen to be $\phi(f_N) = \mu(f; f_N)$, which simplifies the calculation as compared to taking $\phi(f_N) = f_N$. Four examples are calculated for both cases and compared with the original assumed electron density profiles. Accuracy and required computer times are discussed.

INTRODUCTION

The virtual height h′(f) in ionospheric measurements for the evaluation of the electron density profiles is defined (Thomas, 1959) as the height of a sharply bounded reflector, in free space, which would reflect pulses of mean frequency f with time interval t′ between transmission and reflection by the reflector such that $h' = ct'$, where c is the velocity of light in free space and h′ is measured as a function of the frequency of the pulse h′(f). Actually the time delay t′ of the pulse is due to the variation of the refractive index in the ionosphere:

$$t' = \int_0^H \frac{dz}{v_g} \tag{1a}$$

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[1] The Computation Center, University of Kansas, Lawrence, Kansas 66044

[2] Electrical Engineering Department, University of Kansas, Lawrence, Kansas 66044.

where v_g is the group velocity, H is the maximum height beyond which the pulse will not propagate and z is the actual height. By defining the group refractive index $\mu' = c/v_g$ one has from (1a):

$$h' = \int_0^H \mu' dz \tag{1b}$$

The analysis of the ionospheric h′(f) records for the ordinary wave with static magnetic field and no collisions requires the solution of the integral equation (1b) written in the form (Budden, 1961):

$$h'(f) = \int_{f_N = 0}^{f_N = f} \mu'(f; f_N) \frac{dz(f_N)}{d\phi(f_N)} d\phi(f_N) \tag{2}$$

where f_N is the plasma frequency, $z(f_N)$ is the actual height as a function of the plasma frequency (the electron density profile of the ionosphere) and $\phi(f_N)$ is arbitrary single valued function. In the integral equation (2) the curve of virtual height h′(f) is measured, the ionospheric electron profile distribution $z(f_N)$ is the unknown monotonically increasing function to be determined, the group refractive index $\mu'(f; f_N)$ is the kernel of the integral equation and $\phi(f_N)$ is an arbitrary single valued auxiliary function. Thomas (1959) discussed the different methods for solution of the integral equation (2), where different authors used different functions $\phi(f_N)$; Budden (1954, 1961) used $\phi(f_N) = f_N$, King (1957, 1960) used $\phi(f_N) = \ln(f_N/f)$, Paul (1960) used $\phi(f_N) = f^2_N$, Titheridge (1961b) used $\phi(f_N) = 1/f_N$, Paul and Wright (1963) used $\phi(f_N) = \ln(f^2_N)$, and Knecht *et. al.* (1962) used $\phi(f_N) =$ polynomial in f_N.

The relationship between the wave refractive index $\mu(f; f_N)$ and the group refractive index $\mu'(f; f_N)$ as based on their definition, is given by (Ratcliffe, 1959; Budden, 1961):

$$\mu'(f; f_N) = \frac{\partial}{\partial f} [f\mu(f; f_N)] \tag{3}$$

where $\mu = c/v_p$ and $\mu' = c/v_g$, v_p being the phase velocity and v_g being the group velocity of the wave; the partial derivative in (3) means that the plasma frequency f_N and the other parameters such as the magnetic field are kept constant.

The integral equation (2) for the isotropic ionosphere with no magnetostatic field may be solved in a closed form (Appleton, 1930; De Groot, 1930; Manning, 1947; Unz, 1961b) by using Schlömilch's integral equation (Unz, 1963a, 1966; Gething and Maliphant, 1967). Titheridge (1961a) and Unz (1961b, 1962a) suggested expansion in a power series for the solution of the general integral equation (2). This method has been extended by Unz (1962b) for finding electron density profiles in the outer ionosphere. The reduction of ionograms from topside sounders has been discussed by Doupnik and Schmerling (1965) and by Thomas, et. al., (Frihagen, 1966).

The most widely used numerical method for the solution of the integral equation (2) has been the lamination method. This method seems to have been developed first by Murray and Hoag (1937), but the importance of this principle was not realized for almost 20 years. Recently the uniform lamination method has been developed in detail by Budden (1954, 1961) and was extended to nonuniform lamination by Unz (1963b).

The above methods require evaluation of the group refractive index $\mu'(f; f_N)$ from the wave refractive index $\mu(f; f_N)$ and several simplifying methods for doing it have been suggested (Unz, 1961a; Murty and Khastgir, 1962). Alternative methods of solution to avoid the evaluation of the group refractive index have been suggested by Titheridge (1959a, b) and King (1960).

Recently Hojo (1961) and independently Unz (1962a) derived a new integral equation instead of (2), where the wave refractive index μ is used as the kernel function instead of the group refractive index μ'. By substituting (3) into (2) and rearranging, Unz (1962a) obtained:

$$h'_{av}(f) = \frac{1}{f}\int_{0}^{f} h'(f)\,df = \int_{f_N=0}^{f_N=f} \mu(f;f_N)\,\frac{dz(f_N)}{d\phi(f_N)}\,d\phi\,(f_N) \tag{4}$$

where $h'_{av}(f)$ is the average virtual height and is defined as the phase height (Budden, 1961). Equation (4) is the new simplified integral equation and some of its advantages have been pointed out by Titheridge (1963) and by Unz (1964). The integral equation (4) was used to develop a nonuniform lamination analysis method (Unz, 1963b). The integral equations (2) and (4) are known as the Volterra integral equations of the third kind (Tricomi, 1957).

In the present paper we suggest use of the arbitrary single valued function, $\phi(f_N) = \mu(f; f_N)$, in the integral equation (4) and use of the uniform lamination method (Budden, 1954, 1961) in order to solve it. Four different electron density profile curves are assumed: a. linear, b. parabolic, c. sinusoidal, d. rotated sine. The virtual height $h'(f)$ curve and the average virtual height $h'_{av}(f)$ curve for each one are calculated. In each case the uniform lamination method is used to calculate the corresponding electron density profile, and this calculated electron density profile is compared with the original profile. The lamination method is used twice for each curve, once with $\phi(f_N) = f_N$ and once with $\phi(f_N) = \mu(f; f_N)$, and the two results are compared both in accuracy and computer time required. By assuming different electron density profiles the accuracy of each method could be found.

THE LAMINATION METHOD

In the lamination method one may subdivide the interval (0, f) in (4) into n small intervals similarly to Budden (1954, 1961):

(5a)

$$h'_{av}(f) = \sum_{m=1}^{n} \int_{f_N = f_{m-1}}^{f_m} \mu(f;f_N) \frac{dz(f_N)}{d\phi(f_N)} \, d\phi(f_N)$$

where $f_0 = 0$ and $(f_m - f_{m-1})$ will represent small equal intervals for uniform laminations. By using the second mean value theorem in integrals (Goodstein, 1948; Apostol, 1957) one obtains:

(5b)

$$\int_{f_N = f_{m-1}}^{f_m} \mu(f;f_N) \frac{dz(f_N)}{d\phi(f_N)} \, d\phi(f_N) = \left. \frac{dz(f_N)}{d\phi(f_N)} \right|_{f_N = c_m} \int_{f_N = f_{m-1}}^{f_m} \mu(f;f_N) \, d\phi(f_N)$$

where $f_{m-1} < c_m < f_m$

Equation (5b) is exact, except that it is almost impossible to find c_m in general. However, if the intervals $(f_m - f_{m-1})$ are small enough, one is able to approximate:

(5c)

$$\left. \frac{dz(f_N)}{d\phi(f_N)} \right|_{f_N = c_m} \simeq \frac{z(f_m) - z(f_{m-1})}{\phi(f_m) - \phi(f_{m-1})} = \frac{z_m - z_{m-1}}{\phi_m - \phi_{m-1}} = \frac{\Delta z_m}{\Delta \phi_m}$$

The results of (5) are similar to the ones given by Budden (1954, 1961).

Rewriting (5a) for different values $f = f_n$, substituting (5c) into (5b) and the result into (5a) and rearranging, one is able to write a progressive solution in the following form (Budden, 1954, 1961) taking $z_0 = 0$.

(6a)

$$\Delta z_n = \frac{1}{M_{nn}} \left[h'_{av_n} - \sum_{m=1}^{m=n-1} M_{n,m} \Delta z_m \right]$$

where $h'_{av_n} = h'_{av}(f_n)$, $z_n = z_{n-1} + \Delta z_n$ and

(6b)

$$M_{n,m} = \frac{1}{\phi_m - \phi_{m-1}} \int_{f_N = f_{m-1}}^{f_N = f_m} \mu(f_n;f_N) \, d\phi(f_N) \quad \text{for } m \leq n$$

where $M_{n,m} = 0$ for $m > n$

One of the difficulties of applying the lamination method using any of the forms of $\phi(f_N)$ that have been suggested in the past is that $M_{n,m}$ as given in (6b) must be evaluated by some numerical integration technique. This difficulty can be avoided by noting that the form of ϕ which reduces the integral in (6b) to its simplest form is:

$$\phi(f_N) = \mu(f_n;f_N) \tag{7}$$

Substituting (7) into (6b) one has:

$$M_{n,m} = \frac{1}{2}\,[\mu(f_n;f_m) + \mu(f_n;f_{m-1})] \qquad \text{for } m<n \tag{8a}$$

$$M_{n,n} = \frac{1}{2}\;\mu(f_n,f_{n-1}) \qquad \text{for } m = n \tag{8b}$$

$$M_{n,m} = 0 \text{ for } m>n. \tag{8c}$$

Since $\mu(f_n;f_n) = 0$ for the ordinary wave.
The result (8) has been found previously by an alternative approach (Unz, 1963b).

Substituting (8) into (6a) yields the following form:

$$z_n = z_{n-1} + \Delta z_n \tag{9a}$$

$$\Delta z_n = \frac{1}{\mu(f_n;f_{n-1})}\left\{2h'_{av_n} - \sum_{m=1}^{m=n-1}[\mu(f_n;f_m) + \mu(f_n;f_{m-1})]\Delta z_m\right\} \tag{9b}$$

By using (4) and the integral first mean value theorem one obtains:

$$h'_{av_n} = \int_{f_N=0}^{f_n} \mu(f_n;f_N)\;dz(f_N) \cong \sum_{m=1}^{n} \overline{\mu}_m \Delta z_m \cong$$

$$\cong \sum_{m=1}^{n-1} \frac{1}{2}\,(\mu_{m-1} + \mu_m)\;\Delta z_m + A\mu_{n-1}\Delta z_n \tag{9c}$$

where $\mu_m = \mu(f_n;f_m)$ and $\overline{\mu}_m$ is the average value of μ_m in the interval $(f_{m-1};f_m)$; the last term in (9c) is due to the fact that $\mu_n = \mu(f_n;f_n) = 0$ for the ordinary wave and the value of A depends on the approximation used in the last interval (Paul, 1966):

$$A = \frac{1}{2} \text{ assuming } \frac{dz}{d\mu} = \text{const.} \tag{10a}$$

$$A = \frac{2}{3} \text{ assuming height varies linearly with } f^2_N. \tag{10b}$$

$$A = \frac{\sqrt{2}}{2} \text{ taking the mean value of } \mu \text{ in the middle of the interval}$$

$$\text{on the } X = f^2_N/f^2 \text{ scale.} \tag{10c}$$

The result (9b) used in the present paper may be derived directly from (9c) by taking $A = \frac{1}{2}$.

Using the progressive solution (9a) and (9b) one is able to reduce the virtual height records with only the aid of a desk calculator.

THE VIRTUAL HEIGHTS

A comparison of the accuracy and ease of application of the lamination method using $\phi = \mu(f; f_N)$ and $\phi = f_N$ was determined by reducing the virtual height curves from four assumed electron distributions and comparing the results. The assumed electron distributions were linear, parabolic, sinusoidal, and a rotated sine as specified below:

I. linear

$$\frac{f_N^2}{\beta^2} = \alpha z \tag{11}$$

where α and β are normalizing constants.

II. parabolic

$$\left(\frac{f_N}{f_p}\right)^2 = 1 - \left(\frac{z - z_m}{a}\right)^2 \tag{12}$$

where f_p is the penetration frequency, z_M is the height of maximum electron density and "a" is a normalizing constant, commonly denoted as the half-thickness of the layer.

III. sinusoidal

$$\left(\frac{f_N}{f_p}\right)^2 = \sin\left(\frac{\pi z}{2 z_M}\right) \tag{13}$$

IV. rotated sine

$$\left(\frac{f_N}{\alpha}\right)^2 \sin \Theta = \frac{z}{\beta} \cos \Theta + \sin\left\{ \gamma \left[\left(\frac{f_N}{\alpha}\right)^2 \cos \Theta + \frac{z}{\beta} \sin \Theta \right] \right\} \tag{14}$$

where α is normalizing constant for the plasma frequency; β is normalizing constant for the real height; θ is angle through which the coordinate axis is rotated (see Figure 1); and γ is a parameter used to control the maximum value of the slope of the rotated sine curve. The values of the parameters used in the present work are $\theta = 45°$ and $\gamma = 0.8$.

Virtual height curves were calculated from each of these assumed electron distributions (Becker, 1960) as described by the following equations:

$$h'(f) = 2 \int_{t=0}^{t=1} \mu' t \frac{dz}{dX}\, dt \tag{15}$$

where: $t^2 = 1 - \dfrac{f_N{}^2}{f^2} = 1 - X$ and $X = \dfrac{f^2{}_N}{f^2}$.

In order to apply this method it was necessary to evaluate $\dfrac{dz}{dX}$, which was accomplished for each distribution by noting that:

$$\frac{dz}{dX} = f^2 \frac{dz}{df_N^2} \tag{16}$$

and one obtains for the different cases from (16):

$$\text{I.} \quad \text{linear} \quad \frac{dz}{dX} = \frac{f^2}{\alpha \beta^2} \tag{17a}$$

$$\text{II.} \quad \text{parabolic} \quad \frac{dz}{dX} = - \frac{a\ f^2}{2 f_p^2 \sqrt{1 - \dfrac{f_N^2}{f_p^2}}} \tag{17b}$$

(17c)

$$\text{III. sinusoidal} \quad \frac{dz}{dX} = \frac{2f^2 z_M}{\pi f_p^2 \sqrt{1 - \left(\frac{f_N^2}{f_p^2}\right)^2}}$$

IV. rotated sine — The rotated sine electron distribution was specified in the original x′ – y′ coordinate system by:

(18a)

$$y' = \sin (x' + \pi)$$

Rotating the x′ – y′ coordinate system through an angle θ yields the relationships shown in Figure 1. To obtain values of the slope of this curve at uniform intervals on the x coordinate axis, the following procedure was followed.

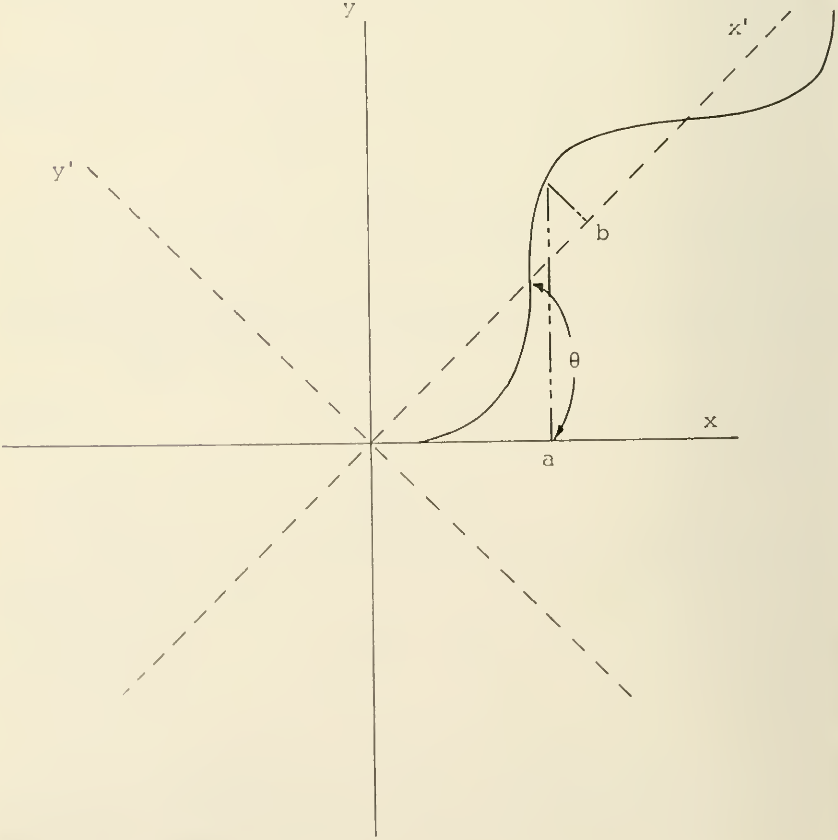

FIG. 1: Rotated Sine Geometry.

First the points on the x axis where the function was to be evaluated were specified. In Figure 1 a typical such point is labeled a. Next the corresponding point on the x′ axis, labeled b, was determined by a Newton-Raphson iteration technique. Knowing point b in the x′ − y′ coordinate system permitted the determination of the slope of the curve in the x′ − y′ coordinate system by the relation:

$$\frac{dy'}{dx'} = \cos(x' + \pi) = \tan\phi \tag{18b}$$

Use was then made of the fact that the slope in the new coordinate system is $\tan(\phi + \theta)$. Thus the quantity $\frac{dz}{dX}$ was evaluated from the following equation:

$$\frac{dz}{dX} = f^2 \tan(\phi + \Theta) = f^2 \frac{\tan\phi + \tan\Theta}{1 - \tan\phi \tan\Theta} \tag{18c}$$

The virtual height as specified by (15) was evaluated at uniformly spaced points for each of the electron distributions by a trapezoidal integration formula, and the effect of the number of points used was determined by making calculations with 10 and 50 point integration formulas. It was found that for the linear, parabolic, and sinusoidal distributions the differences in resulting virtual heights were in the fourth significant digit, and differences in the virtual height of the rotated sine distributions were in the second significant figure. This strong dependency of the rotated sine virtual height upon the number of points can be attributed to the inaccuracy in the iteration procedure that is used to affect the rotation of the coordinate system. In all further calculations the 50 point trapezoidal integration formula was used so that the virtual height curves would not appreciably distort the comparison between the two methods.

All calculations in this comparison were made with normalized heights and frequencies as specified in (11)-(14) and with normalized gyromagnetic frequency of 0. 7448 and dip angle of 40°. Normalization of the gyromagnetic frequency was accomplished by the same method that is used to normalize the plasma frequency in each electron profile. The virtual heights used in the comparison are tabulated in Tables 1, 2, 3, 4.

Virtual heights of the linear, parabolic and sinusoidal electron distributions appear quite smooth and generally possess anticipated characteristics such as for every increase in the real height curve there is an even sharper increase in the virtual height curve. Some significant inaccuracy apparently enters the calculations for the virtual height of the rotated sine curve as shown by the fuzzy section of the virtual height curve near the normalized frequency value of 3.4.

It is important that these inaccuracies in the virtual height curves be noted, since the purpose of these calculations is to compare the accuracy of the methods of reduction of virtual height curves to real height curves. In application to actual recorded data the errors noted above do not appear. However, these inaccuracies in the virtual height curves do provide a useful means of ascertaining the dependence of each method of reduction upon the smoothness of the virtual height data.

The application of (6) to the reduction of the virtual height curves requires that the average of the virtual height up to each frequency be determined. This average was calculated by applying a trapezoidal integration formula where the number of points used in the formula was dependent upon the frequencies at which the virtual height values were available. For example, a 15 point trapezoidal integration formula was used to determine the average virtual height value at the fifteenth frequency where virtual height calculations were made. The same average virtual height (or phase height) was used for calculations in both methods $\phi = f_N$ and $\phi = \mu$. It should be pointed out that for experimental data the average virtual height might be found by adding an integrator to the circuit and dividing the result by the corresponding frequency.

In order to apply (6) with $\phi = f_N$ it was necessary to evaluate the integral in the expression for $M_{n,m}$ in Equation (6b) by a numerical approximation. The approximation chosen was a trapezoidal integration formula, and calculations were made with formulas of 10, 20, and 30 points. It was found that the increase in the number of points from 10 to 20 affected the fourth significant digit, while the increase from 20 to 30 points affected only the fifth significant digit. As a compromise between accuracy and computer time, all further calculations were made using a trapezoidal integration formula of 20 points to evaluate $M_{n,m}$ for $\phi = f_N$.

RESULTS

Table 1 presents the results of reducing the virtual height of a linear electron density profile tabulated at 20 points by using both $\phi = f_N$ and $\phi = \mu$. The results of the reduction are tabulated in the columns labeled calculated real height, and the tabulated percent errors were calculated as follows:

$$\text{percent error} = \frac{(\text{Real Height} - \text{Calculated Real Height})}{\text{Real Height}} \times 100 \qquad (19)$$

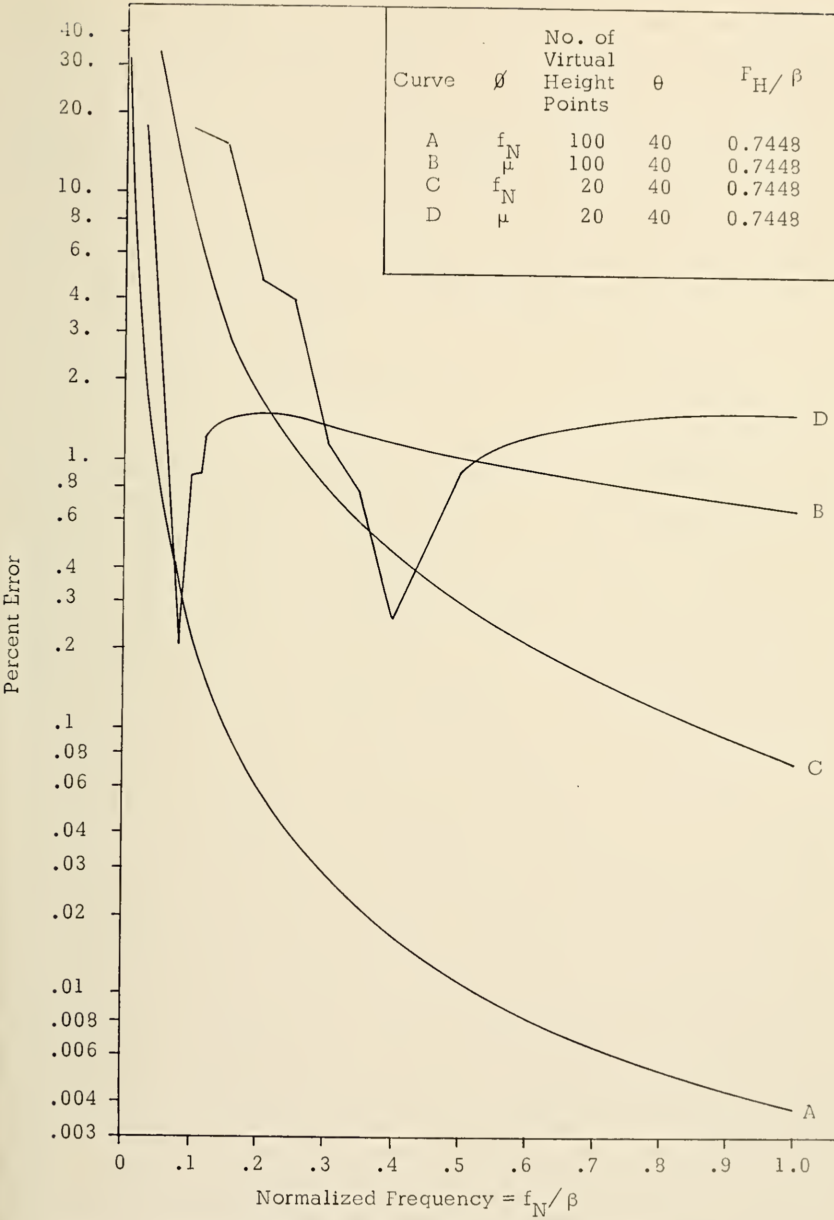

FIG. 2: Error Curves of the Reduction of a Linear Electron Density Profile.

Figure 2 contains curves of the percent error of the reduction of both a 20 point and a 100 point virtual height curve of a linear electron density profile. Curves labeled C and D correspond to the values in Table 1 for a 20 point virtual height curve, and curves A and B are the results from the reduction of a 100 point virtual height curve. Curves A and C represent the error in reduction when $\phi = f_N$ and curves B and D when $\phi = \mu$.

The curves of Figure 2 show that the error in both methods is of the same magnitude at the lower frequencies. The dominant factor contributing to this large error at these low frequencies is probably due to the inaccuracy in $h'_{av}(f)$ calculated values when only a few points are available with which to make the calculations. Thus the low frequency error of reducing the 100 point virtual height curve by either method is considerably less than the error of reducing the 20 point virtual height curve, because at the same frequency more points have been available for calculating $h'_{av}(f)$. Therefore the errors in the low frequency range do not tell us much about the ϕ assumption for the two methods.

Comparing A with B and C with D in Figure 2 shows that the method with $\phi = f_N$ is about 10 times more accurate then the method with $\phi = \mu$. Also evident is the dependence of the f_N method and the independence of the μ method on the number of points at which the virtual height curve is tabulated. In fact the μ method gives almost a constant error of about 1.5 percent.

Table 2 presents the results of the application of the f_N and μ methods to a 20 point virtual height curve of a parabolic electron density profile. For the normalized frequency $f/f_p = 1.00$ the virtual height h' is infinite and the real height is 1.0000. The corresponding numbers given in Table 2 by the computer (without any changes) are 49.1136 and 0.9996 which are acceptable within our range of error. For this case the error in the calculated real height in both methods is quite large; it seems that the only way to rectify it is by calculating h'_{av} over the last few intervals by a higher order integration method than the trapezoidal rule (Paul, 1966). The change from positive errors to negative errors indicates that the two curves are crossing each other.

Figure 3 contains curves of the percent error of the reduction of a 20 and 100 point virtual height curve of a parabolic electron density profile. Curves labeled C and D correspond to the error values tabularized in Table 2, and the A and B curves correspond to the 100 point virtual height curve reduction errors. Again, curves A and C are errors in the f_N method, and B and D errors in the μ method. The same features evident in Figure 2 are also prominent here: (a) the dependency of the error at the low frequencies upon $h'_{av}(f)$; (b) the greater accuracy of the f_N method over the μ method; (c) the dependency of the f_N method on the number of virtual height values used in the analysis; (d) the independence of the μ method on the number of virtual height values. It means that for the μ method other sources of error are dominant.

Results of the reduction of a 20 point sinusoidal profile virtual height curve by both methods are presented in Table 3 and Figure 4, curves C and

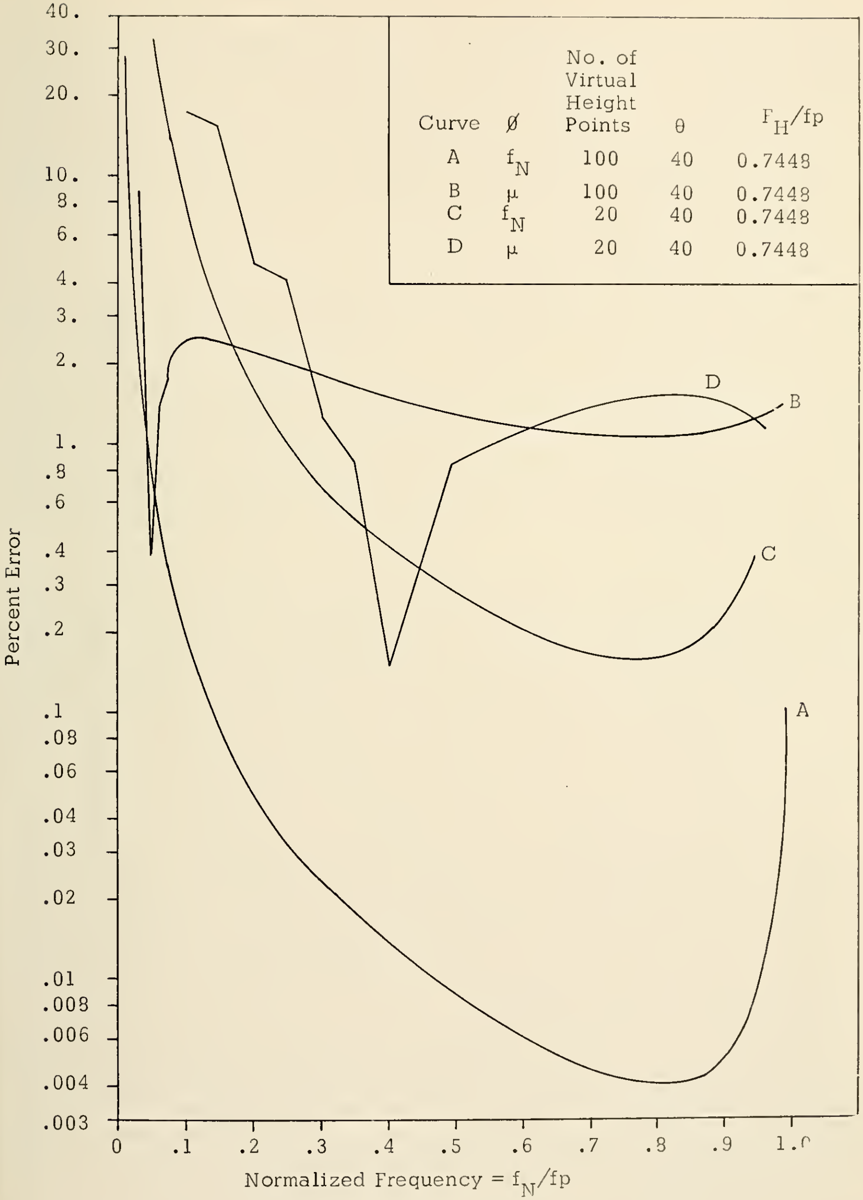

Fig. 3: Error Curves of the Reduction of a Parabolic Electron Density Profile.

D. Also shown in Figure 4 are the results from the reduction of a sinusoidal virtual height curve tabulated at 50 frequencies; the conclusions for the linear and parabolic results also apply to these results.

Table 4 and Figures 5 and 6 present the results of application of the two methods to an 80 point virtual height curve of a rotated sine electron density profile. Figure 5 is the error in the f_N results, and Figure 6 is the error in the μ results. Again both sets of error curves have the same magnitude of error at the low frequencies. The independence of the μ method and dependence of the f_N method upon the number of points at which virtual height points are tabulated is apparently disguised in this set of results by the inaccuracies in the virtual height curve.

It can be seen from the previous discussion and results that the errors in the method where $\phi = f_N$ generally are largest at the low frequencies and continually diminish as the number of virtual height points included in the analysis increases. Some of the decrease in percent error for high plasma frequencies f_N reflects the increase in real height in (19). The linear, parabolic and sinusoidal results show that when the virtual height is known to a high degree of accuracy the percent error is dependent almost entirely upon the number of virtual height measurements used to evaluate the calculated real height.

There are two factors which manifest themselves in the percent error results of the application of the $\phi = \mu$ method. The first error is attributed to the number of virtual height measurements used to evaluate the calculated real height. This error, dominant at the low frequencies and a minimum near the highest frequencies, also is present in the results of the application of the $\phi = f_N$ method. The second error is due to the inherent inaccuracy in the μ method causing about a 1.5 percent error in the calculated real heights.

Observation of the percent error values for the rotated sine where the values for the virtual heights could not be obtained accurately because of the difficulty in calculating the slope of the real height curves, indicate that neither method of analysis is critically dependent upon the smoothness of the virtual height curves. This attribute can be explained by noting that the methods of reduction do not use the raw virtual heights directly, but use instead the average of the virtual heights.

Although the f_N method is inherently more accurate than the μ method, the application of the f_N method requires a far greater number of calculations than the μ method. For example, Table 5 gives the number of minutes required to make the indicated reduction by each of these methods on an IBM 7040 digital computer.

As has been pointed out, the prime source of error in both methods at the low frequencies is attributed to the inaccuracy in the $h'_{av}(f)$ values. These values could have been determined analytically exactly, and this error source can be avoided. But, in an actual application of either of these methods, this would not have been possible when reducing a measured virtual height curve.

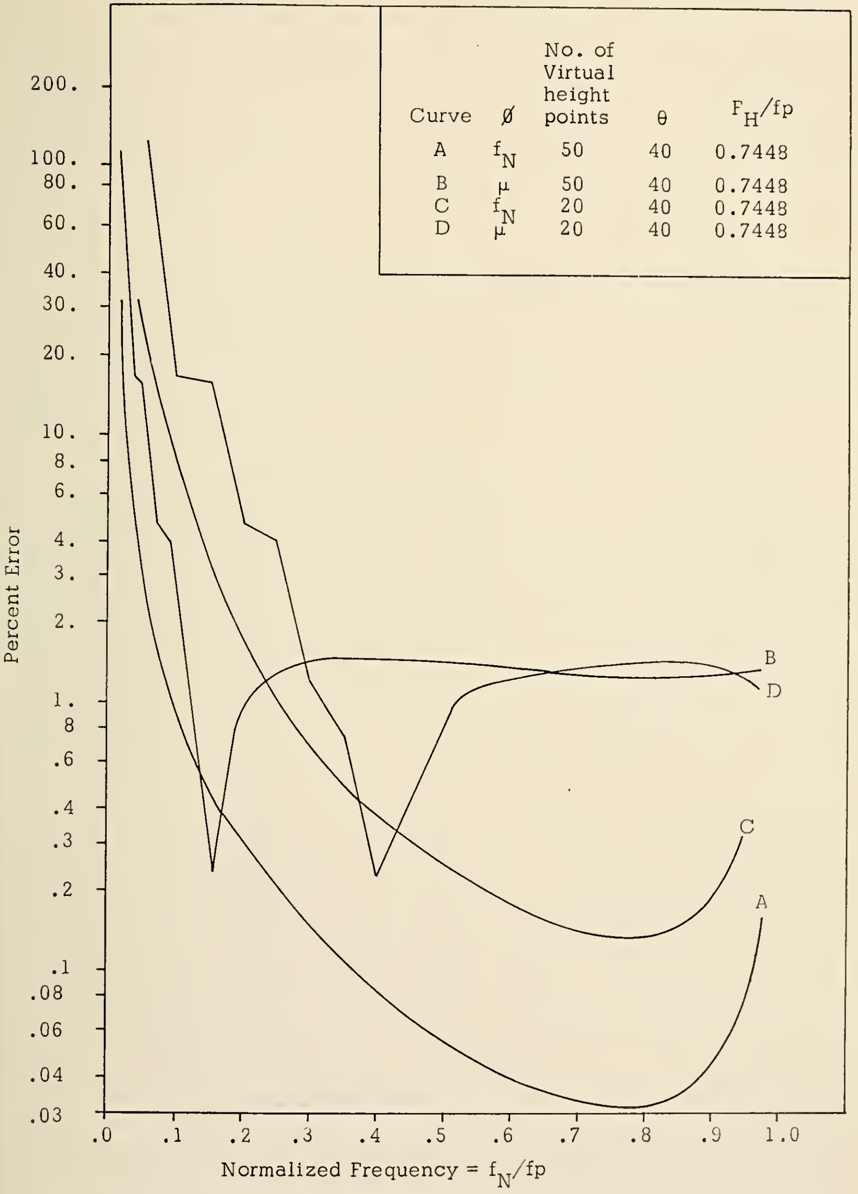

FIG. 4: Error Curves of the Reduction of a Sinusoidal Electron Density Profile.

Thus it is important to leave this error source in the calculations so that the effect of this error could be evaluated.

One of the difficulties of previous methods of analysis has been that in

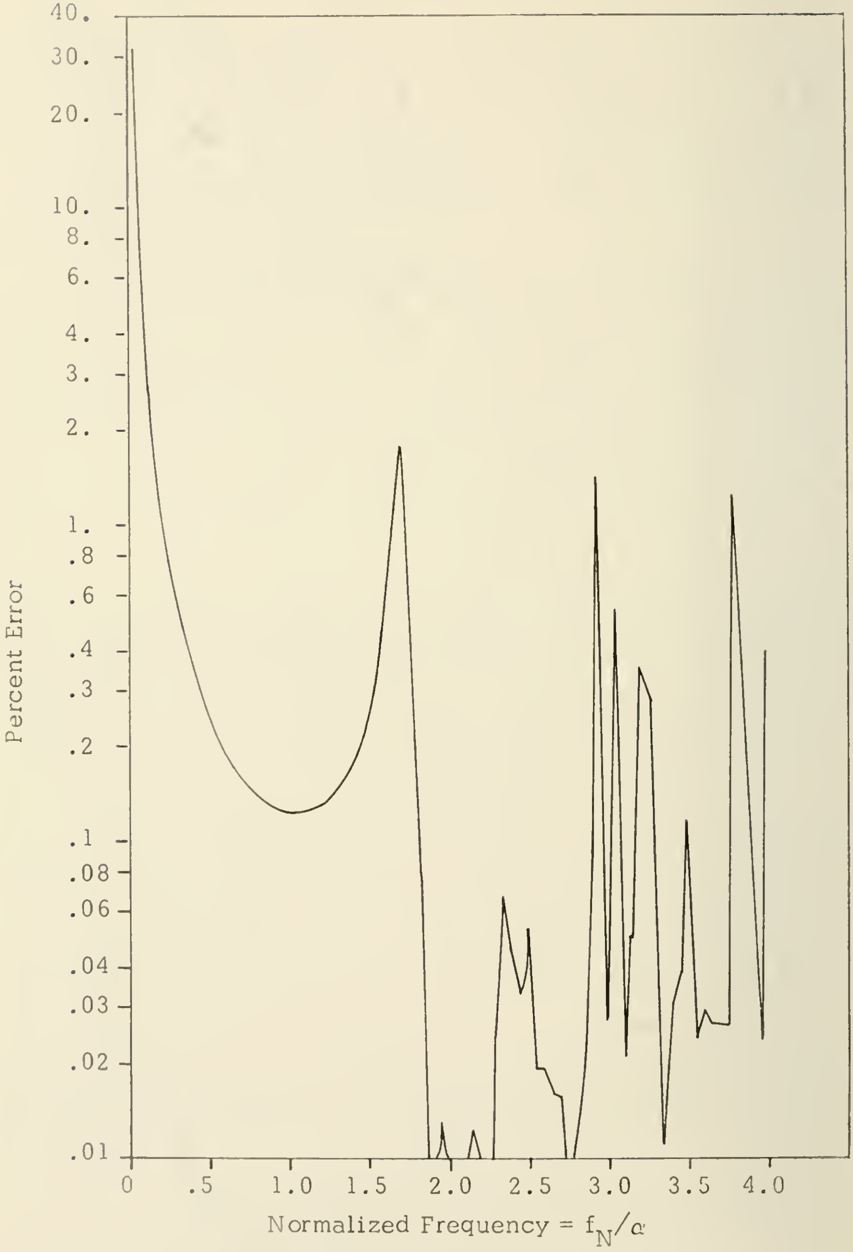

FIG. 5: Error of the Reduction by $\phi = f_N$ of the Virtual Height of a Rotated Sine Profile.

order to apply the method by hand it was necessary that virtual height measurements be made at certain prescribed frequencies because of the difficulty of evaluating such constants as M_{nm} in (6b). Normally to apply the method by hand it was necessary to use a table of values of M_{nm}. The method prescribed by $\phi = \mu$ enables one to be independent of any precalculated tables, thus permitting the freedom to make the virtual height measurements at any frequency.

The time necessary to apply the μ method by hand was determined by reducing a virtual height curve that was tabulated at ten points with a static magnetic field assumed to be zero. The calculations were made in 90 minutes with a desk calculator and a table of square roots of integer numbers. This time could have been reduced to about forty-five minutes if a table of generalized values of the refractive index (Becker, 1960) was used.

SUMMARY AND DISCUSSION

The uniform lamination analysis method is used for the solution of the simplified integral equation which relates the electron density profiles with the average virtual heights, taking the wave refractive index as the kernel function. The arbitrary auxiliary function is chosen to be $\phi(f_N) = f_N$ and $\phi(f_N) = \mu(f; f_N)$. Four examples are calculated for both cases, and the results are compared with the original assumed electron density profiles. The accuracy and the required computer times are discussed. The $\phi = \mu$ method though less accurate than the $\phi = f_N$ method, is especially suitable for calculations by hand.

The aim of the present paper has been to investigate the errors resulting from the analysis of ionospheric records, by taking four different, monotonically increasing, assumed electron density profiles. Both methods in their present form are only applicable to monotonic profiles (Paul and Wright, 1963). The accuracy of the $\phi = \mu$ method might be improved (Paul, 1966) by taking different A in (9c) and it will have the added advantage of much less computing time. A more detailed analysis and further study of the different errors inherent in the present approach will be necessary before this method can be recommended for practical application.

The present method of using $h'_{av}(f)$ instead of $h'(f)$ might be criticized because of reducing the method's sensitivity to information derived from the ionosphere. On the other hand, the integrated effect might conceivably reduce the effect of local inhomogeneities in the ionosphere as well as the instruments' error in each particular measurement by taking integrated values. The smoothing effect on the calculated $h'(f)$ curves when transformed to $h'_{av}(f)$ curves was noticed by the authors. The present method concentrates on improving the computing speed and reducing the cost, while maintaining a reasonable accuracy.

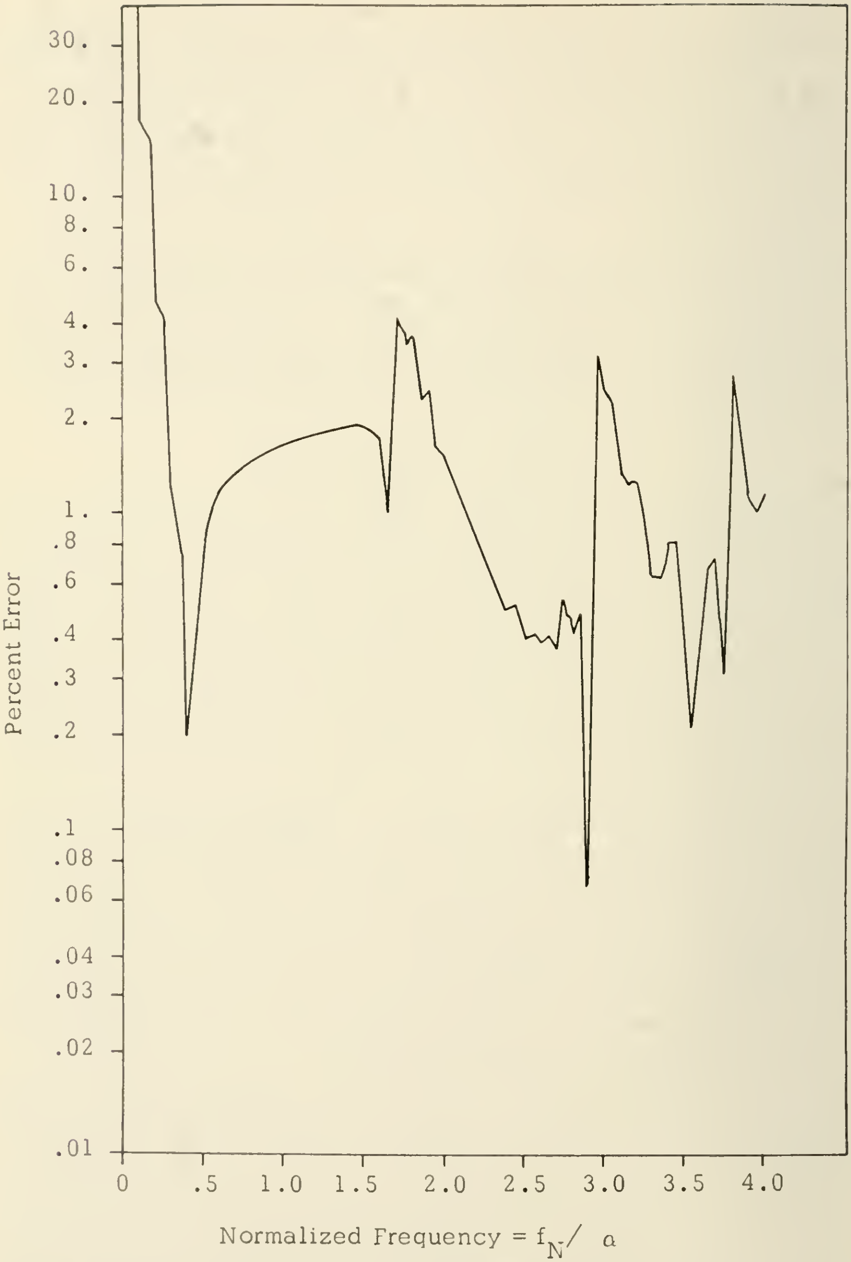

FIG. 6: Error of the Reduction by $\phi = \mu$ of the Virtual Height of a Rotated Sine Profile.

Several different methods for the analysis of ionospheric profiles have been suggested and are presently in use. It is hoped that other workers in the field will use their methods for calculating the electron density profiles suggested in the present paper in order to find the corresponding errors and the required computer time. By using the presently suggested test, the accuracy of and the computer time required by each method could be ascertained and the different methods of calculation could be compared by a standard test.

ACKNOWLEDGMENT

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The calculations in the present paper have been done on the IBM 7040 electronic digital computer at the Computation Center, University of Kansas, Lawrence, Kansas.

This is also to thank Mrs. Lynda Austin for typing the manuscript.

Table 1

Linear Profile

Norm. Freq.	Virtual Height	Real Height	$\Phi = \mu$ Calc. Real Height	$\Phi = f_N$ Calc. Real Height	$\Phi = \mu$ Per Cent Error	$\Phi = f_N$ Per Cent Error
0.00	0.0000	0.0000	0.0000	0.0000	0.000	0.00
0.05	0.0055	0.0025	0.0055	0.0033	–120.350	–32.779
0.10	0.0220	0.0100	0.0117	0.0105	–17.543	–5.930
0.15	0.0495	0.0225	0.0258	0.0231	–15.030	–2.787
0.20	0.0879	0.0400	0.0418	0.0406	–4.683	–1.519
0.25	0.1373	0.0625	0.0649	0.0631	–3.954	–0.973
0.30	0.1975	0.0900	0.0910	0.0906	–1.172	–0.670
0.35	0.2686	0.1225	0.1234	0.1231	–0.757	–0.492
0.40	0.3504	0.1600	0.1596	0.1606	0.242	–0.376
0.45	0.4429	0.2025	0.2015	0.2031	0.490	–0.297
0.50	0.5461	0.2500	0.2477	0.2506	0.907	–0.240
0.55	0.6600	0.3025	0.2993	0.3031	1.051	–0.199
0.60	0.7844	0.3600	0.3555	0.3606	1.239	–0.167
0.65	0.9194	0.4225	0.4169	0.4231	1.319	–0.142
0.70	1.0649	0.4900	0.4831	0.4906	1.405	–0.123
0.75	1.2209	0.5625	0.5543	0.5631	1.446	–0.107
0.80	1.3874	0.6400	0.6305	0.6406	1.484	–0.094
0.85	1.5643	0.7225	0.7116	0.7231	1.500	–0.084
0.90	1.7517	0.8100	0.7977	0.8106	1.512	–0.075
0.95	1.9494	0.9025	0.8888	0.9031	1.514	–0.067
1.00	2.1575	1.0000	0.9848	1.0006	1.513	–0.061

Table 2

Parabolic Profile

Norm. Freq.	Virtual Height	Real Height	$\Phi = \mu$ Calc. Real Height	$\Phi = f_N$ Calc. Real Height	$\Phi = \mu$ Per Cent Error	$\Phi = f_N$ Per Cent Error
0.00	0.0000	0.0000	0.0000	0.0000	0.000	0.000
0.05	0.0027	0.0012	0.0027	0.0016	–120.403	–32.811
0.10	0.0110	0.0050	0.0059	0.0053	–17.653	–5.965
0.15	0.0249	0.0113	0.0130	0.0116	–15.113	–2.818
0.20	0.0446	0.0202	0.0211	0.0205	–4.796	–1.548
0.25	0.0702	0.0317	0.0330	0.0320	–4.043	–1.001
0.30	0.1020	0.0460	0.0466	0.0463	–1.281	–0.698
0.35	0.1405	0.0632	0.0637	0.0635	–0.848	–0.520
0.40	0.1860	0.0834	0.0833	0.0838	0.142	–0.404
0.45	0.2394	0.1069	0.1065	0.1073	0.404	–0.326
0.50	0.3013	0.1339	0.1328	0.1343	0.820	–0.271
0.55	0.3731	0.1648	0.1632	0.1652	0.977	–0.231
0.60	0.4564	0.2000	0.1976	0.2004	1.170	–0.202
0.65	0.5532	0.2400	0.2370	0.2405	1.262	–0.181
0.70	0.6671	0.2858	0.2819	0.2863	1.356	–0.167
0.75	0.8031	0.3385	0.3338	0.3391	1.406	–0.158
0.80	0.9696	0.4000	0.3942	0.4006	1.447	–0.158
0.85	1.1824	0.4732	0.4663	0.4740	1.457	–0.169
0.90	1.4767	0.5641	0.5560	0.5663	1.423	–0.210
0.95	1.9639	0.6877	0.6795	0.6903	1.189	–0.382
1.00	49.1136	0.9996	5.5210	5.2256	–452.282	–422.733

TABLE 3
Sinusoidal Profile

Norm. Freq.	Virtual Height	Real Height	$\Phi = \mu$ Calc. Real Height	$\Phi = f_N$ Calc. Real Height	$\Phi = \mu$ Per Cent Error	$\Phi = f_N$ Per Cent Error
0.00	0.0000	0.0000	0.0000	0.0000	0.000	0.000
0.05	0.0035	0.0015	0.0035	0.0021	−120.348	−32.778
0.10	0.0140	0.0063	0.0074	0.0067	−17.544	−5.930
0.15	0.0315	0.0143	0.0164	0.0147	−15.040	−2.788
0.20	0.0560	0.0254	0.0266	0.0258	−4.689	−1.521
0.25	0.0875	0.0398	0.0413	0.0402	−3.963	−0.976
0.30	0.1260	0.0573	0.0580	0.0577	−1.185	−0.674
0.35	0.1717	0.0781	0.0787	0.0785	−0.773	−0.497
0.40	0.2247	0.1023	0.1020	0.1026	0.220	−0.383
0.45	0.2854	0.1298	0.1292	0.1302	0.465	−0.305
0.50	0.3541	0.1608	0.1594	0.1612	0.876	−0.251
0.55	0.4318	0.1956	0.1936	0.1960	1.018	−0.212
0.60	0.5195	0.2344	0.2316	0.2348	1.202	−0.183
0.65	0.6189	0.2776	0.2741	0.2781	1.280	−0.162
0.70	0.7326	0.3260	0.3215	0.3264	1.364	−0.148
0.75	0.8649	0.3803	0.3749	0.3808	1.403	−0.139
0.80	1.0229	0.4421	0.4357	0.4427	1.435	−0.137
0.85	1.2202	0.5140	0.5066	0.5147	1.438	−0.146
0.90	1.4878	0.6010	0.5926	0.6021	1.405	−0.181
0.95	1.9242	0.7165	0.7079	0.7189	1.199	−0.330
1.00	41.4445	0.9997	4.7758	4.5305	−377.726	−353.189

TABLE 4

Rotated Sine Profile

Norm. Freq.	Virtual Height	Real Height	$\Phi = \mu$ Calc. Real Height	$\Phi = f_N$ Calc. Real Height	$\Phi = \mu$ Per Cent Error	$\Phi = f_N$ Per Cent Error
0.00	0.0000	0.0000	0.0000	0.0000	0.000	0.000
0.05	0.0005	0.0002	0.0005	0.0003	−120.351	−32.779
0.10	0.0020	0.0009	0.0011	0.0009	−17.546	−5.931
0.15	0.0046	0.0021	0.0024	0.0021	−15.043	−2.789
0.20	0.0082	0.0037	0.0039	0.0038	−4.695	−1.523
0.25	0.0128	0.0058	0.0060	0.0059	−3.971	−0.979
0.30	0.0185	0.0084	0.0085	0.0084	−1.199	−0.678
0.35	0.0253	0.0115	0.0116	0.0115	−0.790	−0.503
0.40	0.0332	0.0150	0.0150	0.0151	0.198	−0.389
0.45	0.0424	0.0192	0.0191	0.0192	0.440	−0.313
0.50	0.0529	0.0238	0.0236	0.0239	0.848	0.260
0.55	0.0650	0.0291	0.0288	0.0292	0.990	−0.222
0.60	0.0790	0.0351	0.0347	0.0352	1.175	−0.194
0.65	0.0952	0.0419	0.0414	0.0420	1.259	−0.174
0.70	0.1140	0.0496	0.0489	0.0497	1.252	−0.159
0.75	0.1361	0.0584	0.0576	0.0585	1.405	−0.148
0.80	0.1624	0.0685	0.0675	0.0686	1.460	−0.139
0.85	0.1937	0.0801	0.0789	0.0802	1.501	−0.133
0.90	0.2314	0.0936	0.0922	0.0937	1.543	−0.129
0.95	0.2772	0.1094	0.1077	0.1096	1.581	−0.127
1.00	0.3330	0.1280	0.1260	0.1282	1.621	−0.125
1.05	0.4017	0.1501	0.1477	0.1503	1.661	−0.125
1.10	0.4868	0.1766	0.1736	0.1768	1.703	−0.126
1.15	0.5929	0.2084	0.2047	0.2087	1.746	−0.127
1.20	0.7264	0.2470	0.2426	0.2473	1.790	−0.120
1.25	0.8959	0.2943	0.2889	0.2947	1.833	−0.134
1.30	1.1139	0.3527	0.3461	0.3532	1.876	−0.141
1.35	1.3988	0.4259	0.4178	0.4266	1.917	−0.150
1.40	1.7794	0.5191	0.5089	0.5199	1.953	−0.164
1.45	2.3037	0.6402	0.6275	0.6414	1.978	−0.187
1.50	3.0601	0.8027	0.7867	0.8045	1.982	−0.227
1.55	4.2346	1.0317	1.0118	1.0349	1.931	−0.306
1.60	6.3100	1.3837	1.3559	1.3906	1.716	−0.500
1.65	10.9587	2.0250	2.0046	2.0459	1.007	−1.025
1:70	17.9262	3.2055	3.0715	3.1488	4.182	1.767
1.75	16.6461	3.9918	3.8516	4.0210	3.510	−0.731
1.80	14.6571	4.4111	4.2520	4.4050	3.608	0.138
1.85	13.1309	4.6813	4.5701	4.6851	2.376	−0.379
1.90	11.9972	4.8726	4.7629	4.8723	2.249	0.005
1.95	11.0849	5.0145	4.9306	5.0152	1.673	−0.013
2.00	10.3778	5.1266	5.0430	5.1228	1.555	−0.004
2.05	9.7985	5.2062	5.1410	5.2064	1.252	−0.003

TABLE 4 (continued)
Rotated Sine Profile

Norm. Freq.	Virtual Height	Real Height	$\Phi = \mu$ Calc. Real Height	$\Phi = f_N$ Calc. Real Height	$\Phi = \mu$ Per Cent Error	$\Phi = f_N$ Per Cent Error
2.10	9.3270	5.2712	5.2111	5.2716	1.141	–0.006
2.15	8.9228	5.3221	5.2713	5.3228	0.955	–0.012
2.20	8.5783	5.3622	5.3154	5.3625	0.872	–0.005
2.25	8.2952	5.3943	5.3534	5.3944	0.755	–0.003
2.30	8.0874	5.4207	5.3847	5.4220	0.663	–0.024
2.35	7.9122	5.4438	5.4142	5.4475	0.544	–0.066
2.40	7.7297	5.4661	5.4377	5.4685	0.519	–0.044
2.45	7.6307	5.4902	5.4608	5.4884	0.535	0.032
2.50	7.7579	5.5191	5.4968	5.5220	0.404	–0.054
2.55	7.7005	5.5566	5.5319	5.5576	0.443	–0.019
2.60	8.0566	5.6078	5.5853	5.6089	0.401	–0.019
2.65	8.3063	5.6800	5.6556	5.6810	0.430	–0.016
2.70	9.1353	5.7845	5.7617	5.7879	0.395	–0.058
2.75	9.9546	5.9407	5.9095	5.9405	0.524	0.002
2.80	12.2483	6.1872	6.1601	6.1953	0.438	–0.131
2.85	15.8716	6.6252	6.5920	6.1953	0.438	–0.131
2.90	29.5812	7.7102	7.7155	7.7767	–0.068	–0.862
2.95	36.5534	9.4170	9.1130	9.2891	3.228	1.358
3.00	30.3106	10.0431	9.7905	10.0458	2.515	–0.027
3.05	26.5910	10.3639	10.1357	10.3096	2.201	0.524
3.10	24.3320	10.5592	10.4151	10.5569	1.364	0.021
3.15	21.0174	10.6861	10.5539	10.6808	1.237	0.049
3.20	19.8141	10.7711	10.6347	10.7328	1.266	0.355
3.25	19.1085	10.8293	10.7151	10.7976	1.055	0.292
3.30	18.5373	10.8711	10.8026	10.8765	0.630	–0.049
3.35	17.2644	10.9044	10.8357	10.9057	0.630	–0.011
3.40	16.4576	10.9366	10.8465	10.9031	0.823	0.305
3.45	16.4081	10.9753	10.8833	10.9315	0.838	0.398
3.50	17.0830	11.0300	10.9707	11.0169	0.537	0.119
3.55	17.6481	11.1142	11.0909	11.1407	0.210	–0.238
3.60	17.6709	11.2500	11.1999	11.2534	0.445	–0.029
3.65	19.7160	11.4808	11.4014	11.4500	0.692	0.268
3.70	24.4993	11.9925	11.8327	11.8912	0.752	0.262
3.75	43.7194	13.1848	13.1439	13.2190	0.310	–0.259
3.80	51.4629	15.1159	14.7077	14.9298	2.700	1.231
3.85	43.7207	15.7037	15.4511	15.7491	1.608	–0.289
3.90	37.7636	15.9892	15.7993	16.0048	1.187	–0.097
3.95	33.3169	16.1516	15.9819	16.1476	1.050	0.024
4.00	29.9946	16.2494	16.0538	16.1845	1.203	0.399

Table 5

Comparison of the Time of Reduction by $\phi = f_x$ and $\phi = \mu$ On An I.B.M. 7040

No. of Virtual Height Points	Time of Reduction for $\Phi = f_x$	Time of Reduction for $\phi = \mu$
11	.0739 Min.	.0167 Min.
21	.2533 Min.	.0336 Min.
65	8.1992 Min.	.4381 Min.

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THE EFFECT OF ALTERATION OF TECHNIQUE AT TWO STAGES IN A NUMERICAL TAXONOMIC STUDY

By

Theodore J. Crovello

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The Effect of Alteration of Technique at Two Stages in a Numerical Taxonomic Study

Theodore J. Crovello[1]
Department of Entomology, The University of Kansas, Lawrence

ABSTRACT

The effect of change of technique at two stages of a numerical taxonomic study was investigated. This investigation is based on data from 30 taxospecies of *Salix,* willow, for 131 morphological characters. The first stage studied was that designed to remove unequal weighting of characters caused by measurement of different characters on different scales. Here standardization was compared with condensation. The second stage analyzed was the formation of the basic data matrix, a character by taxon table. One-dimensional character state distance was compared with two-dimensional character state distance. In the former, only an estimate of central tendency (the mean) was used. In the latter both the mean and an estimate of variation (the standard deviation) were used. A coefficient of Euclidean distance was calculated and clustering was performed both by the unweighted pair group method employing averages and by principal components analyses of the similarity matrices. Each of the four combinations of methods produced some clusters that were the same in each analysis. But many differences were apparent. Change of technique at both stages appears to result in different relationships.

The Pearson product moment correlation coefficient does not appear to be a sensitive statistic for judging how well two similarity matrices, two phenograms or a similarity matrix and a phenogram agree with each other. Two phenograms with a correlation of —0.975 still had at least one taxospecies in different clusters.

I. INTRODUCTION

Methods of numerical taxonomy (Sokal and Sneath, 1963) are being used more and more both in the construction of taxonomic systems and as an aid in biosystematic work. But many users are unaware that there is not just one

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[1] Present address: Department of Biology, University of Notre Dame, Notre Dame, Indiana.

method of numerical taxonomy. Although it has one purpose, a substantial number of different techniques are in use that achieve this purpose. Unfortunately, no absolute test exists for deciding which of the methods is best, in whatever way a systematist chooses to define best.

The purpose of this paper is to assay the effect of change of technique at two stages in a numerical taxonomic study. The first stage is the formation of the taxon by character basic data matrix (BDM). The first method is one that takes intrataxon variation of a character into account (two-dimensional character state distance), while the second method does not (one-dimensional character state distance). The second stage involves the comparison of two methods of transforming characters to relieve them of the unequal weighting introduced by the use of different units of measurement (the widely used method of standardization with the less well known technique of condensation).

Although previous workers such as Minkoff (1965), Boyce (1964) and Sokal and Michener (1967) have investigated the effect of change of technique at such stages as clustering, I know of no other study that has analyzed effects of change of technique at the two stages currently being considered.

II. MATERIALS AND METHODS

As a byproduct of a study of the pattern of variation among members of section *Sitchenses* of the genus *Salix,* Crovello (1966) accumulated information on the 30 taxospecies of the genus in California. Table 1 lists the taxospecies and their codes. For comparative purposes they are arranged into sections according to Schneider (1921), the last monographer of the group.

The 131 characters used in the present study are as follows: *Last year's twig characters*—twig color; twig length; twig width; flakiness of twig; twig pruinose. *Last year's bud characters*—bud color; bud length (dehisced flower buds); bud appressed to twig; bud scale open or fused; bud apex shape. *Leaf characters, except pubescence*—stipules present or absent; stipule length; stipule margin; stipule margin glandular; petiole length; petiole glandular; blade length; blade shape; blade margin; blade margin glandular; blade margin revolute; blade base angle; blade apex angle; veins prominent below; color of veins below; abaxial side glaucous; adaxial side of blade lustre; number of leaves from the tip; veins protruding below abaxial surface; stipule width; stipule shape. *Habit characters*—habit; height of plant. *This year's twig characters*—length of twig; number of leaves on twig; twig diameter; flakiness; pruinose; color of twig. *Female characters*—number of aments per lateral branch; ament length; ament width; peduncle length from ament to first leaf; overall peduncle length; peduncle leaf number; peduncle leaf length; peduncle leaf width; peduncle leaf margin; direction of flowering within the ament; ament dense or lax; rachis with bumps like spruce twig;

floral scale length; scale shape; scale color; scale margin; scales persistent or deciduous; number of veins on distal end of scale; adaxial nectary number; adaxial nectary shape; adaxial nectary length; adaxial nectary width; abaxial nectary number; abaxial nectary shape; abaxial nectary length; abaxial nectary width; scale pubescent on entire adaxial surface; scale pubescent on entire abaxial surface; stigma lobe length; stigma lobe number; style length; capsule length; capsule width; capsule pedicel length; stigmas revolute; seed length (embryo length); seed coat length; seed width (embryo width); cotyledon length; seed hair length. *General pubescence characters*—pubescence on last year's twigs; pubescence on female floral buds; pubescence on this year's vegetative buds; pubescence on abaxial leaf surface; pubescence on adaxial leaf surface; pubescence on this year's twig; pubescence on female peduncle leaf; pubescence on female rachis; pubescence on adaxial side of female floral scale; pubescence on abaxial side of female floral scale; pubescence on capsule surface. *Male characters*—number of aments per lateral branch; ament length; ament width; peduncle length from ament to first leaf; overall peduncle length; peduncle leaf number; peduncle leaf length; peduncle leaf width; peduncle leaf margin; direction of flowering within the ament; ament dense or lax; rachis with bumps like spruce twig; floral scale length; scale shape; scale color; scale margin; scales persistent or deciduous; number of veins on distal end of scale; adaxial nectary number; adaxial nectary shape; adaxial nectary length; adaxial nectary width; abaxial nectary number; abaxial nectary shape; abaxial nectary length; abaxial nectary width; scale pubescent on entire adaxial surface; scale pubescent on entire abaxial surface; stamen number; anther length; anther color; filament length; filaments divided; pollen length; percent pollen stained in lactophenol. *General male pubescence characters*—pubescence on stamen filaments, pubescence on flower bud; pubescence on the peduncle leaf; pubescence on the rachis; pubescence on adaxial side of floral scale; pubescence on abaxial side of floral scale.

Whenever possible characters were not coded. Thus, for continuous characters such as leaf length the raw measurements were used to indicate the character state on a plant. The actual method of measuring each character may be found in Crovello (1966). For each taxospecies 6 to 15 plants were selected and at least two measurements were made per character per plant. Operating on one character at a time, the average value was calculated for each plant. The mean and standard deviation for a taxospecies was then calculated from these averages. This resulted in two character by taxospecies data matrices, one containing the mean value for every character in each taxospecies, the other containing the standard deviation. The usual numerical taxonomic study uses only the matrix of means or some other estimate of central tendency.

The availability of a matrix of estimates of variation about the mean makes it possible to include intrataxon variation in a taximetric analysis. This is accomplished by estimating two-dimensional character state distance. The procedure for its estimation can be understood if one envisions that a two-dimensional graph is prepared for each character. One axis expresses the estimate of central tendency for each OTU (operational taxonomic unit; see Sokal and Senath, 1963), while the other represents the estimate of variation within each OTU. The position of an OTU is found by plotting the two values for each OTU, one from each data matrix. Crovello (1966) called this space *character state two-space. Two-dimensional character state distance* (CSD2) is then found by the following formula which is the familiar Pythagorean theorem,

$$\mathrm{CSD2} = [(X_{ij} - X_{ik})^2 + (Y_{ij} - Y_{ik})^2]^{1/2}$$

where X_{ij} and X_{ik} are the values of the mean for character i in OTU's j and k, respectively, and Y_{ij} and Y_{ik} are the values of the standard deviation for character i in OTU's j and k, respectively. A separate value of CSD2 is obtained for each character for every pair of OTU's. This results in a single OTU by character matrix that can be used as a basic data matrix in the conventional numerical taxonomic study. The latter was also used in the present study to serve as a comparison. It is simply the OTU by character matrix of the means of each character. If desired, one can consider this matrix as displaying relationships in a *character state one-space.* As such, no preliminary calculations are necessary. Each value in this matrix may be considered an estimate of *one-dimensional character state distance* (CSD1).

To remove unequal weighting of characters due to differences in the units of measurement used for each, two methods were analyzed in the present study. *Standardization* (Sokal and Sneath, 1963) is the most commonly used method in numerical taxonomy today. But it seemed to me to possess a serious drawback when used in actual taxonomic situations. Because the number of OTU's analyzed in any one study is not infinite, and because the frequency distribution of many characters is not normal, the standardized ranges of characters are not equal. The reader can verify this in the example of standardized values in Sokal and Sneath (1963:296). Standardization has reduced the undesired weighting effect but has not eliminated it.

The results of standardization will be compared with those obtained from *condensation. Condensation* is the name given by Crovello (1966) to a modification of a simple, unnamed linear transformation introduced by Cain and Harrison (1958). Sheals (1964) used the same modification. The condensed value, X_{ci} of some value of character X, X_i, is

$$X_{ci} = \frac{X_i - X_{min}}{X_{max} - X_{min}}$$

where X_{min} is the minimum value of the character in the data matrix and X_{max} is the maximum observed value. Accordingly, the transformed states of each character range from zero to one. It should be obvious that if one were to condense the standardized values, the result would be similar to condensation alone.

The two methods at two stages of a taximetric study can be combined into four basic analyses: (1) condensation of mean values; (2) condensation of values of CSD2; (3) standardization of mean values; and (4) standardization of values of CSD2. In each case, the coefficient of distance used to estimate similarity is the square root of Sokal's (1961) coefficient. When condensation is used the value of the coefficient lies between zero and one, the former indicating absolute similarity and the latter absolute dissimilarity *in the context of the characters used in the analyses.* The usual range of the coefficient when standardization is employed is from about zero to about three. For convenience, in those analyses using condensation each distance coefficient was subtracted from one. This enables a reader to note percent similarity between two OTU's directly. We shall call the resulting tables of distance (or of similarity) the basic similarity matrix (BSM). Four BSM's were obtained, one from each of the four analyses.

Two methods of cluster analysis were used. In the first, a phenogram was computed from each BSM using the average unweighted pair group method (Sokal and Sneath, 1963). In the second method, the correlation matrix was derived from each BSM, and it was subjected to a principal components analysis, which is simply a rigid rotation of axes in the object space of 30 dimensions that can be formed from any of the four 30 by 30 BSM's obtained in the present study. The relationships among the OTU's remain the same, but each principal component axis removes the maximum amount of variation in the 30-dimension object space as reflected in the correlation matrix. For details of the method the interested reader is referred to Seal (1964).

III. RESULTS

The results of the four analyses are presented both graphically (Figs. 1-8) and statistically (Tables 2-4). In all analyses the OTU by OTU relevance (Sokal and Sneath, 1963) had an observed mean of 0.908 and a standard deviation of 0.032 ($n = 435$).

1. *Graphical Comparison*

Figures 1 through 4 present graphic results of the four analyses using the unweighted pair group method (by averages) of clustering. The author visually defined eight clusters in Figure 1. An OTU once assigned to a cluster in Figure 1 keeps that cluster number in subsequent figures. In this way the reader can understand quickly the similarities and differences in clustering among the four phenograms. The first phenogram will be compared to conventional ideas to facilitate understanding of the results. Conventional concepts used here are those of Schneider given in Table 1.

Eight clusters appear in Figure 1, which gives the results using one-dimensional character state distance (CSD1) and condensation. Starting at the top of the figure, the first four OTU's form a definite cluster consisting of the four representatives of the subgenus *Pleiandrae.* All other OTU's are taxospecies of subgenus *Diandrae.* The next cluster contains the four OTU's assigned to section *Longifoliae.* This is followed by another cluster of four taxospecies from section *Cordatae.* But PSCORD and LASLEP, two other representatives of this section according to Schneider, appear in later clusters. Cluster 4 includes six OTU's belonging to five conventional sections. The fifth cluster consists of the two taxospecies each of sections *Brewerianae* (BREWER, DELNRT) and *Sitchenses* (JEPSON, SITCHS). Note that SITCHS appears closer to section *Brewerianae* than it does to the other member of section *Sitchenses.* Cluster 6 contains the two members of section *Chrysanthae* plus LASLEP, placed by Schneider in section *Cordatae.* Recall that cluster 3 consisted of four representatives of section *Cordatae.* As in the previous cluster, a conventional section is split. The seventh cluster consists of three high altitude willows. Two are dwarfed, alpine forms. They have been placed in three sections. Finally, cluster 8 contains mountainous taxospecies. Both are from different taxonomic sections and both sections have a second representative in the present study.

Figures 2 through 4 present phenograms from the other three analyses. The easiest way to compare the four phenograms is to examine the eight clusters with respect to their internal and external structure. All four analyses clearly recognize clusters 1 and 2. Each contains the same OTU's, although within each cluster the order of their grouping differs with each analysis. Some difference in the order of grouping within a cluster was observed in most clusters in each of the analyses. To conserve space, differences within clusters will not be mentioned again until the discussion. Cluster 3 is maintained throughout, except that in analyses 2 and 3 TRACYI is not present. For cluster 4, analysis 3 agrees in content with analysis 1 while both analyses 2 and 4 lack COMUTA and analysis 2 also lacks SCOULR. JEPSON is not present in cluster 5 in any analysis except analysis 1. Clusters 6 and 8 contain the same OTU's in all four studies. For cluster 7, the only difference between analysis 1 and the others is that PLANIF is not present in the other clusters. In these it appears more related to cluster 4. To summarize, let an OTU that appears in analyses 2 through 4 in a cluster different from its placement in analysis 1 be scored as one difference. Then analysis 2 (CSD2, condensation) has 5 differences and analyses 3 (CSD1, standardization) and 4 (CSD2, standardization) each have 3 differences.

Figures 5 through 8 graphically present the results of clustering by principal components. This clustering method must tolerate a dilemma. Potentially, it is more accurate than a phenogram in portraying relationships but such accuracy is harder to comprehend. A phenogram summarizes relationships of 30 dimensions (in the present study) into one, but great distortion is present. A principal components solution accurately summarizes relationships of 30 dimensions in five or six in the present study. It is difficult to see the true pattern of variation when we are confined to views of only three dimensions at a time.

Each of Figures 5 to 8 present the first three component axes of their respective analysis. Each figure consists of two parts, an upper half, a, and a lower half, b. The abscissa is the same in both parts. It represents the first principle component axis. In the upper half of the figure (e.g., Figure 5a) the ordinate depicts the second principal component axis and the lower half (e.g., Figure 5b) depicts the third principal component axis.

All three dimensions are at right angles to each other and the reader should imagine each figure folded 90 degrees halfway up the figure. In this way a view of relationships in three dimensions is obtained. For the four analyses, the first three dimensions accounted for 63, 51, 61 and 51 percent, respectively, of the variation present in the correlation matrices derived from the four basic similarity matrices.

Because comparison of particular OTU's and inner cluster structure in Figures 5 through 8 is more difficult to describe verbally than in previous figures, and to conserve space, only the salient features of the analyses will be considered. The reader interested in more detail has sufficient information to understand the study in as much detail as he desires. The numbers in each figure correspond to the taxospecies code numbers given in Table 1. Numbers are used to minimize bias when examining the figures. Bias is of two sources: (1) preconceived taxonomic ideas, and (2) apparent closeness of six letter codes on a figure when the actual position may not be as close.

In all of the four figures displaying the results of the principal components analyses the same general pattern emerges. The first two axes reveal three large clusters. In each case the two to the left of the origin correspond to clusters 1 and 2 of the phenograms. OTU's to the right of the origin represent the members of clusters 3 to 8 of the phenograms. Note that in the third axis in each analysis the two clusters left of the origin occupy more or less the same position. To obtain a familiarity with the results, the reader should compare the positions in Figures 5 to 8 of the taxospecies of several clusters from Figure 1. In this way he can obtain a graphic idea of the degree of congruence of the different analyses.

2. *Statistical Comparison*

Table 2 presents the mean and standard deviation of the BSM of each of the four analyses. Valid comparisons can be made only between analyses 1 and 2 and between analyses 3 and 4. Within each pair the results are constant, relatively speaking. This might be expected since all analyses used the same set of data. In contrast, a greater difference within each pair was expected because one analysis considered variation about the mean, which variation has not been shown to be homogeneous.

Table 3 presents the Pearson product moment correlation coefficient between all combinations of similarity matrices and phenograms. The upper left quadrant indicates that the four BSM's are very highly correlated. Along with the information from Table 3 this implies that the same relationships are depicted in each BSM. The lower right quadrant contains correlations among the phenograms. These correlations are very high. These two sets of correlation coefficients are both reassuring and distressing. They are reassuring because they indicate that the different techniques used in the four analyses produce the same (highly correlated) results. But they are distressing in that even with such high correlations there exist differences among the analyses. For example, BSM's 2 and 4 are most highly correlated, yet TRACYI is not even in the same cluster in both. As another example, phenograms 2 and 3 show the highest correlation, but SCOULR appears in completely different clusters in the two. These findings indicate a degree of insensitivity of the Pearson correlation coefficient to detect differences be-between BSM's and between phenograms. At the same time they suggest that when a cophenetic correlation coefficient is used to compare a phenogram and its BSM, anything less than |.95| say, probably contains some serious distortions, either within a cluster or between clusters. The cophenetic correlation coefficients appear in the lower left quadrant of Table 3 along the quadrant's principal diagonal. Their range (0.858-0.890) is well below the correlations among BSM's and among phenograms.

Table 4 presents the correlation between the four distance matrices (obtained by using each of the three principal components as characters) and the BSM's, the phenograms, and the other distance matrices. The principal diagonal of the upper third of the table contains the values analogous to the cophenetic correlation coefficient between a BSM and its phenogram. The statistics range in absolute value from 0.842 to 0.870.

IV. DISCUSSION

The results of the present study indicate that the changes in technique investigated here do make a difference in the final taximetric outcome. But the magnitude of difference varies at the different levels of taxonomic organization. A high number of the same clusters were recognized in each analysis, while remaining clusters agreed less among analyses. At this level, numerical taxonomy gave fairly repeatable results under change of technique. Within each cluster, many clusters varied with each analysis both in terms of content of the cluster and of relationships among members within the cluster. One should keep in mind that at all levels of organization, the relationships depicted in the four analyses would have been less if any of the following had occurred: (1) different subsets of characters were used; (2) methods more different from each other were used (e.g., CSD1 and CSD2 always have the same value of central tendency); (3) information for each analysis was obtained from a different sample of the same taxospecies; (4) the amount of missing data for the estimate of variation increased (the CSD2 should diverge more from CSD1).

Some of the patterns of Tables 3 and 4 have not been mentioned previously in the numerical taxonomic literature. These include the fact that in analyses with changes of technique as employed here the correlation among BSM's and among phenograms is higher than the correlation between a BSM and its phenogram. The same may be said for correlations among distance matrices derived from principal component analyses. The correlation between any two such distance matrices is higher than the correlation between such a distance matrix and its original BSM (Table 4).

Finally, we note that the agreement of the results of numerical taxonomy with those of Schneider (1921) is good. Both approaches recognize the subgenus *Pleiandrae* and sections *Longifoliae, Cordatae, Sitchenses, Brewerianae* and *Chrysanthae* as distinct or partially distinct. The major differences involve section *Cordatae* and those unique taxospecies that are the only representatives of their section in California. Section *Cordatae* appears to be too broadly defined. Either it should be restricted to exclude PSCORD and LASLEP or it should be expanded to include other sections. Subsequent study may support the recognition of a supersection or of another subgenus for section *Cordatae* and sections closely related to it.

V. ACKNOWLEDGMENTS

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TABLE 1. The Thirty Taxospecies of *Salix* in California Arranged into Sections According to Schneider (1921).[a]

Taxospecies Number	Taxospecies Code	Taxospecies
section Pentandrae Dumortier		
1	LASAND	*S. lasiandra* Benth.
2	CAUDAT	*S. caudata* (Nutt.) Heller
3	LAEVIG	*S. laevigata* Bebb
section Nigrae Loudon		
4	GOODIG	*S. Gooddingii* Ball
section Longifoliae Andersson		
5	HINDSI	*S. Hindsiana* Benth.
6	EXIGUA	*S. exigua* Nutt.
7	MELANO	*S. melanopsis* Nutt.
8[b]	PARKSI	*S. Parksiana* Ball
section Cordatae Barratt		
9	LUTEA	*S. lutea* Nutt.
10	LIGULI	*S. ligulifolia* (Ball) Ball
11	MACKEN	*S. Mackenziana* (Hook.) Barr.
12	PSCORD	*S. pseudocordata* And.
13	LASLEP	*S. lasiolepis* Benth.
14	TRACYI	*S. Tracyi* Ball
section Adenophyllae Schneider		
15	COMUTA	*S. comutata* Bebb
16	EASTWD	*S. Eastwoodiae* Ckll.
17	ORESTR	*S. orestera* Sch.
section Chrysanthae Koch		
18	PIPERI	*S. Piperi* Bebb
19	HOOKER	*S. Hookeriana* Barr.
section Ovalifoliae Rydberg		
20	ANGLOR	*S. anglorum* Cham. var. *antiplasta* Sch.
section Reticulatae Fries		
21	NIVALI	*S. nivalis* Hook.
section Phylicifoliae Dumortier		
22	PLANIF	*S. planifolia* Pursh. var. *monica* (Bebb) Sch.
23	DRUMSB	*S. Drummondiana* var. *subcoerulea* (Piper) Ball
section Sitchenses Bebb		
24	SITCHS	*S. sitchensis* Sans.
25	JEPSON	*S. Jepsonii* Sch.
section Brewerianae Schneider		
26	BREWER	*S. Breweri* Bebb
27	DELNRT	*S. delnortensis* Sch.
section Discolores Barratt		
28	SCOULR	*S. Scouleriana* Barr.
section Fulvae Barratt		
29	LEMMON	*S. Lemmonii* Bebb
30	GEYERI	*S. Geyeriana* And.

[a] The only exception to Schneider's assignments is *S. Jepsonii.* He placed it in section Phylicifoliae.

[b] Described after 1921, placed in this section by Ball.

TABLE 2. The Mean and Standard Deviation of the Four Similarity Matrices (n = 435 in all cases).

Analysis number and description	Mean	Standard Deviation
1. CSD1, condensed	.617	.077
2. CSD2, condensed	.612	.060
3. CSD1, standardized	1.386	.289
4. CSD2, standardized	1.396	.230

TABLE 3. Pearson Product Moment Correlation Coefficient Between All Combinations of Similarity Matrices and Phenograms.

	Analysis Number	Basic Similarity Matrix				Phenogram			
		1	2	3	4	1	2	3	4
Basic Similarity Matrix	1	1.000							
	2	0.965	1.000						
	3	—0.982	—0.964	1.000					
	4	—0.936	—0.983	0.966	1.000				
Phenogram	1	0.858	0.863	—0.862	—0.849	1.000			
	2	0.848	0.882	—0.866	—0.882	0.942	1.000		
	3	—0.860	—0.880	0.890	0.890	—0.956	—0.975	1.000	
	4	—0.830	—0.876	0.869	0.899	—0.926	—0.955	—0.967	1.000

TABLE 4. Pearson Product Moment Correlation Coefficient Between Distance Matrices Derived from Principal Components Study and Basic Similarity Matrices, Phenograms and Other Principal Components Matrices.

		Principal Components Study			
	Analysis Number	1	2	3	4
Basic Similarity Matrix	1	−0.859	−0.867	−0.863	−0.840
	2	−0.839	−0.870	−0.864	−0.861
	3	0.812	0.835	0.860	0.839
	4	0.781	0.824	0.842	0.842
Phenogram	1	−0.849	−0.876	−0.901	−0.889
	2	−0.838	−0.879	−0.896	−0.901
	3	0.825	0.855	0.895	0.886
	4	0.779	0.825	0.860	0.868
Principal Components	1	1.000			
	2	0.956	1.000		
	3	0.926	0.970	1.000	
	4	0.898	0.974	0.982	1.000

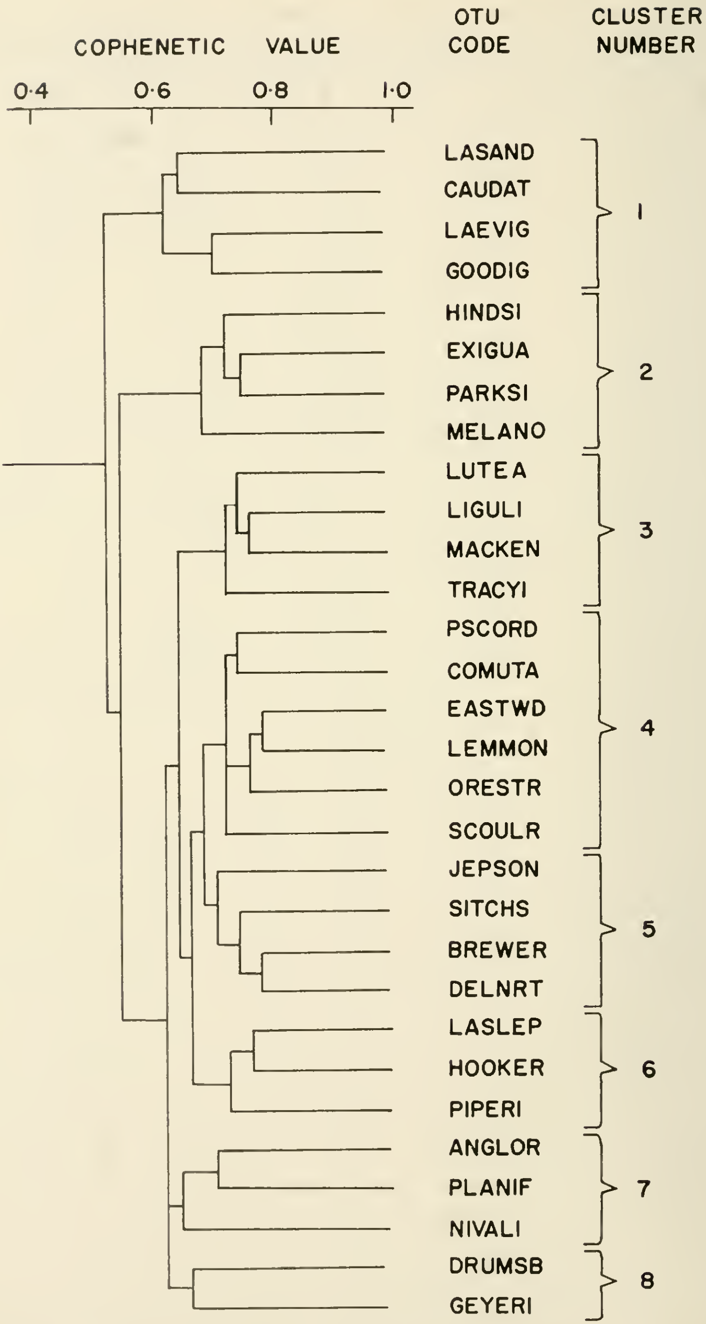

FIGURE 1. Phenogram from analysis one (CSD1, condensation).

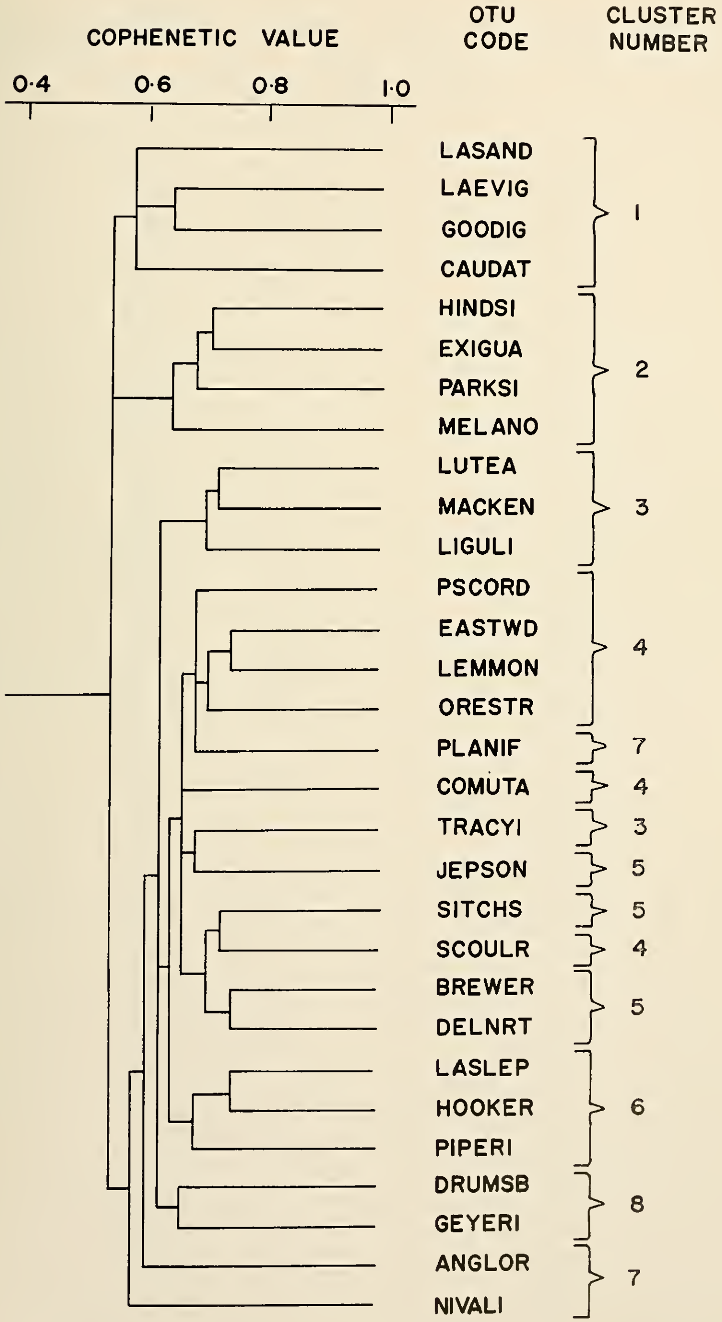

FIGURE 2. Phenogram from analysis two (CSD2, condensation).

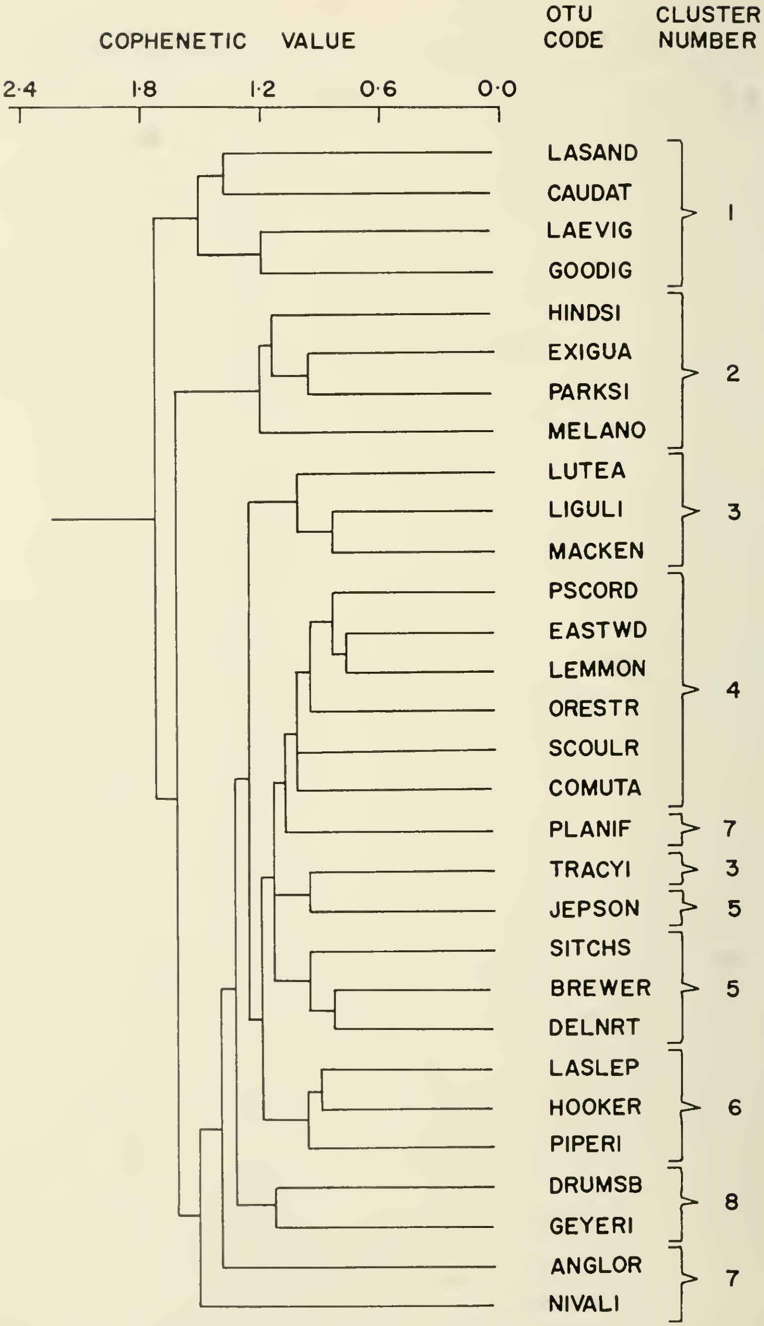

FIGURE 3. Phenogram from analysis three (CSD1, standardization).

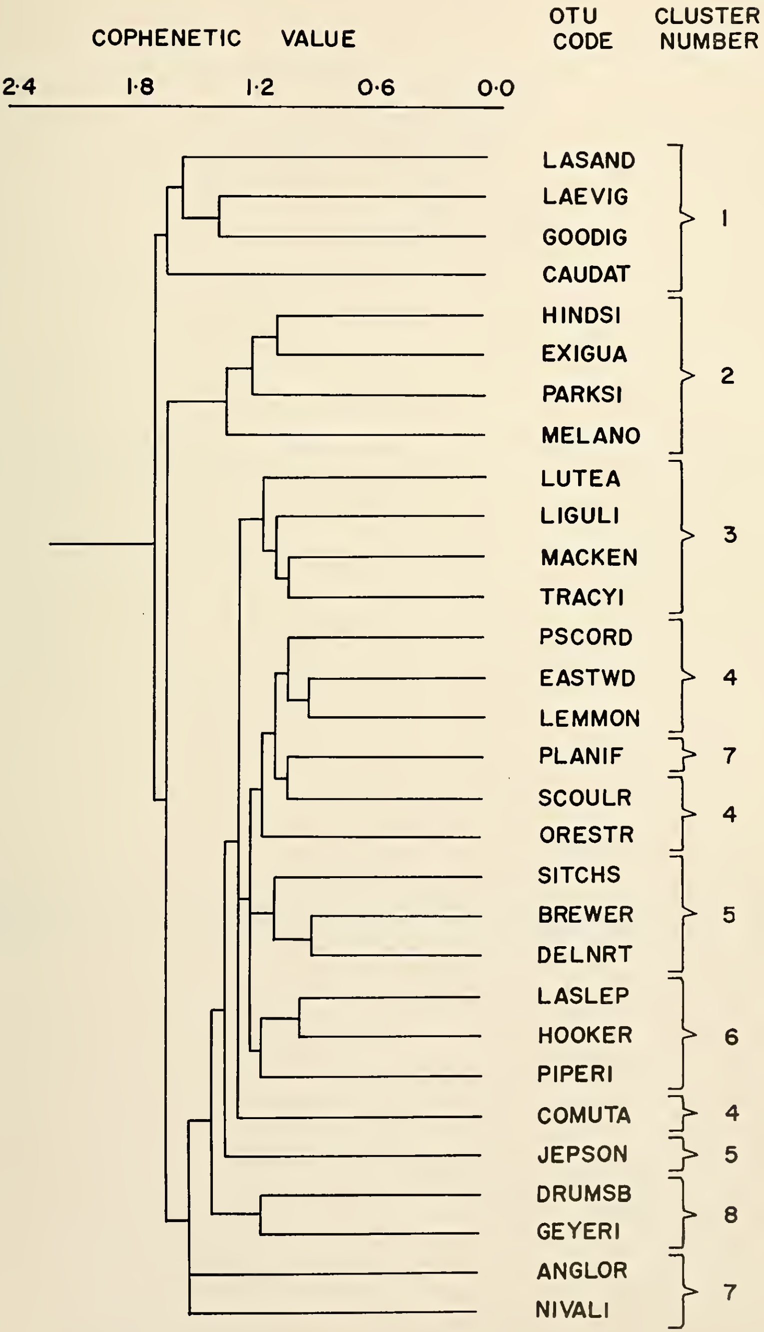

FIGURE 4. Phenogram from analysis four (CSD2, standardization).

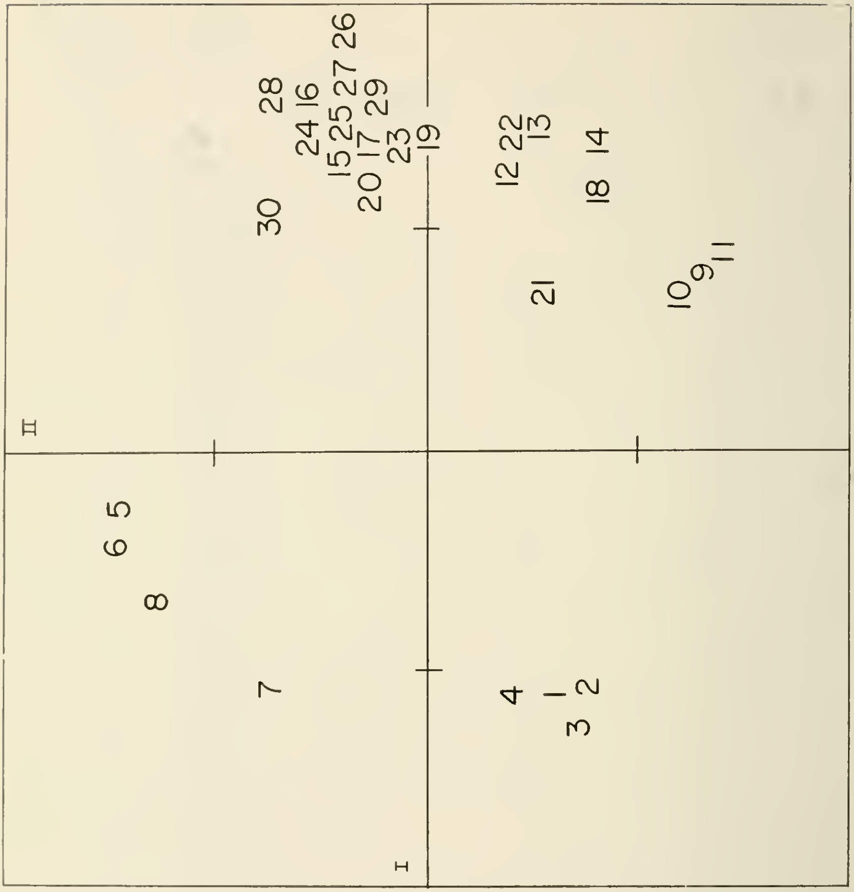

Figure 5a. First two principal components from analysis one (CSD1, condensation). Figure 5a should be viewed with Figure 5b with 5a on top.

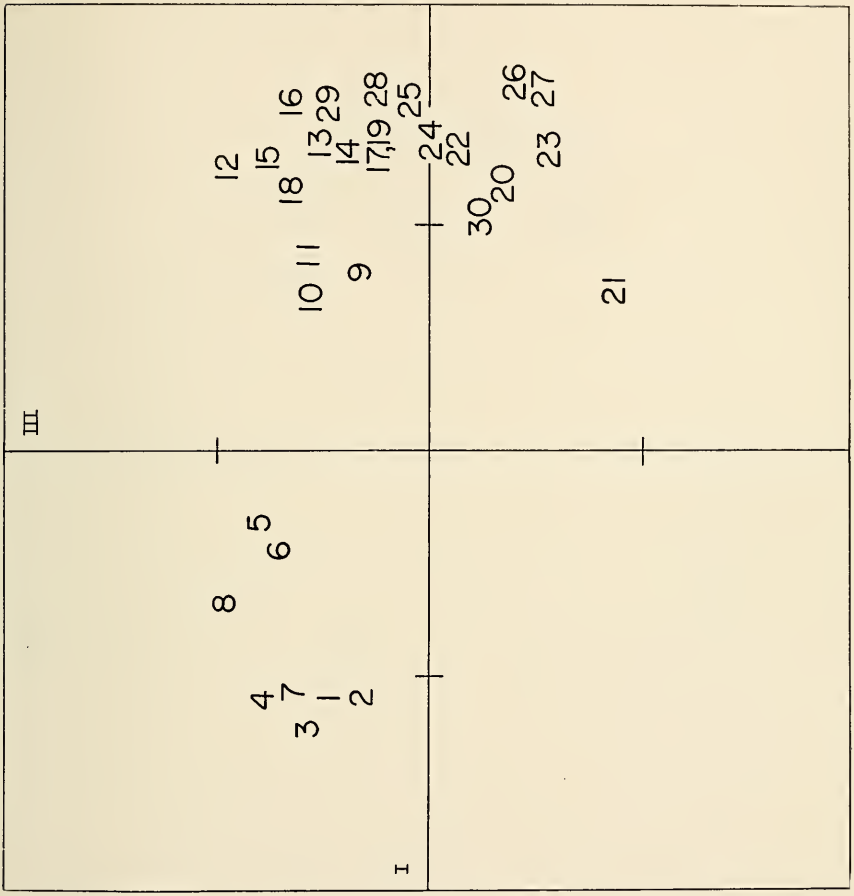

FIGURE 5b. Components one and three from analysis one (CSD1, condensation).

Figure 6a. First two principal components from analysis two (CSD2, condensation). Figure 6a should be viewed with Figure 6b with 6a on top.

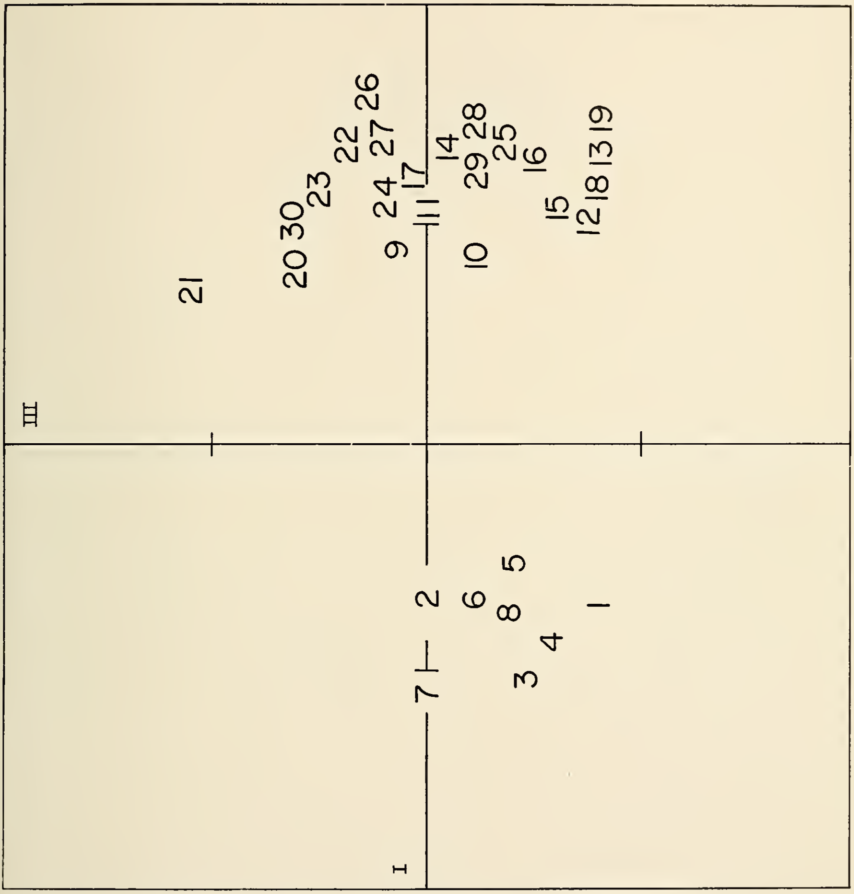

Figure 6b. Components one and three from analysis two (CSD2, condensation).

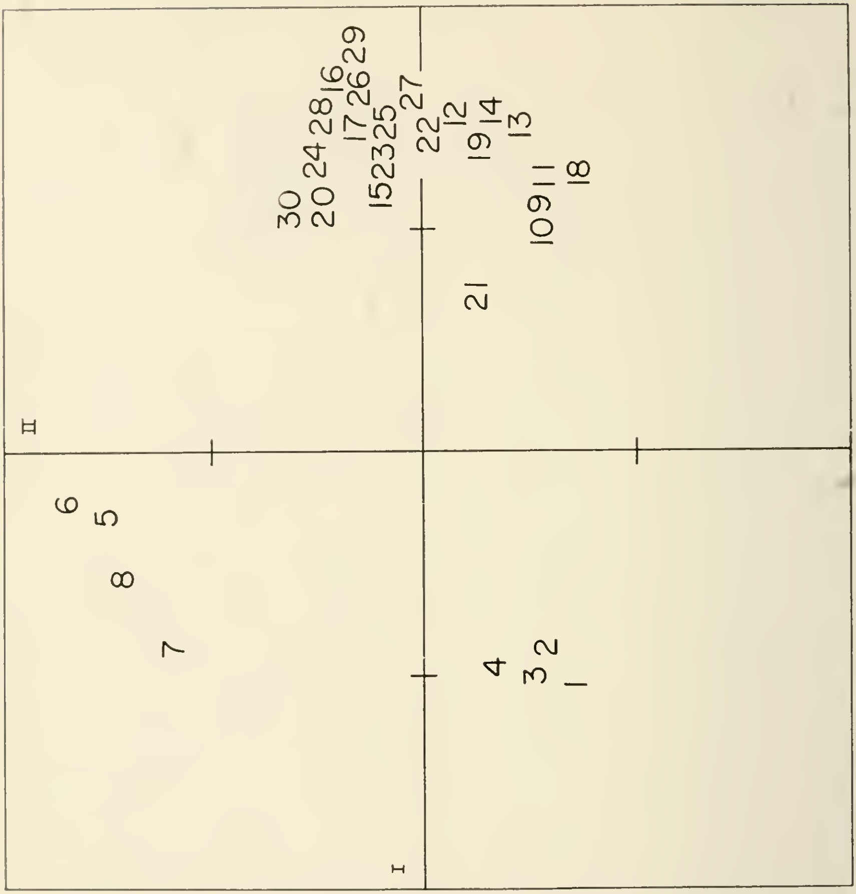

FIGURE 7a. First two principal components from analysis three (CSD1, standardization). Figure 7a should be viewed with Figure 7b with 7a on top.

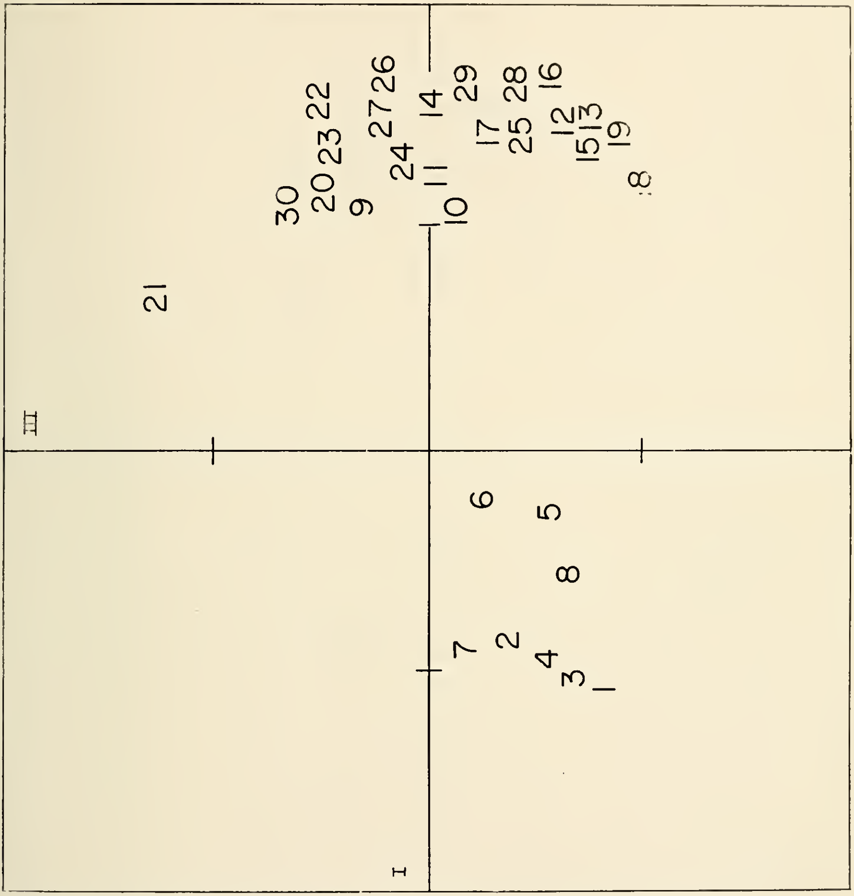

FIGURE 7b. Components one and three from analysis three (CSD1, standardization).

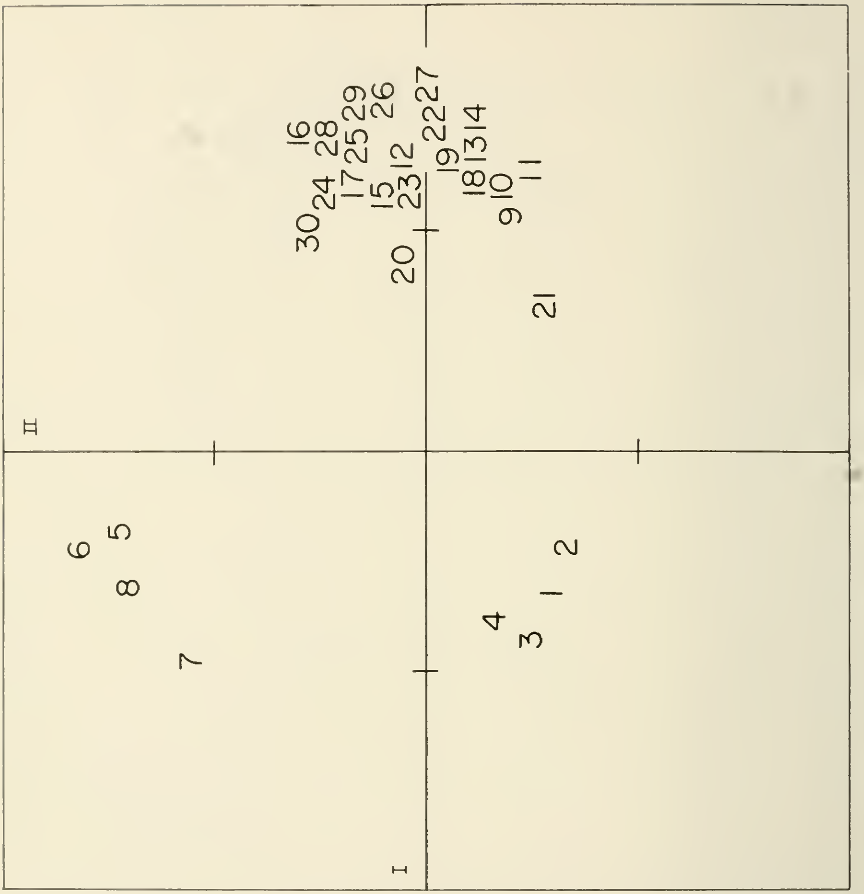

Figure 8a. First two principal components from analysis four (CSD2, standardization). Figure 8a should be viewed with Figure 8b with 8a on top.

Figure 8b. Components one and three from analysis four (CSD2, standardization).

THE UNIVERSITY OF KANSAS
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GEOGRAPHIC VARIATION OF THE RABBIT TICK, *HAEMAPHYSALIS LEPORISPALUSTRIS*, IN NORTH AMERICA

By

Paul A. Thomas

Vol. XLVII Pages 787-828 February 15, 1968 No. 13

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THE UNIVERSITY OF KANSAS
SCIENCE BULLETIN

VOL. XLVII PAGES 787-828 FEBRUARY 15, 1968 No. 13

Geographic Variation of the Rabbit Tick, *Haemaphysalis Leporispalustris,* in North America[1, 2]

PAUL A. THOMAS[3, 4]
Department of Entomology
The University of Kansas

ABSTRACT

The geographic variation of larval, male, and female ticks was summarized and described in terms of four factors. Each factor illustrated a suite of characters and was represented at each locality by the average of standardized scores of the two characters most highly loaded on that factor. The factor scores were plotted on maps for interpretation of patterns of distributions and tested for significant differences by means of the SNK multiple comparisons test. The resulting maps showed various patterns of variation and are illustrated in Figures 2 through 5.

The phenetic similarity of 74 larval tick populations (localities) in a six-dimensional character space was determined by calculating projections of localities on the first three principal axes of the character correlations. Two- and three-dimensional scatter diagrams of localities with respect to three principal axes showed dispersion of localities corresponding to their geographic distribution and were of value in predicting geographic origin of "unknown" specimens and in separating size and shape differences.

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[1] Contribution No. 1357 from the Department of Entomology of The University of Kansas, Lawrence, Kansas. This study represents a portion of a dissertation submitted in partial fulfillment of the requirements for the Ph.D. degree.

[2] This research was supported by a fellowship from the National Institute of General Medical Sciences of the U.S. Public Health Service (Fellowship No. 5-F1-GM-16, 511-03) and by Public Health Service research grant GM 11935 to Robert R. Sokal.

Statistical computations were carried out at The University of Kansas Computation Center.

[3] Present address: The Air Force Armament Laboratory, Eglin AFB, Florida.

[4] I wish to express my appreciation to the following for their assistance in connection with this study: To Dr. Robert R. Sokal for introducing me to statistics and its application in studies of geographic variation and for his advice and encouragement throughout this study; to Dr. Joseph H. Camin for proposing the use of the rabbit tick and for his interest and valuable suggestions; to Dr. Richard Johnston for reading and commenting on the manuscript; to Dr. F. James Rohlf for providing helpful advice on computational methods; to Dr. Glen M. Kohls and many other workers listed in Thomas (1965), who generously loaned tick specimens; to Dr. Harold Willis for photographic assistance; and, finally, to my wife, Judith, for her patience throughout this study and for her assistance with the preparation of the figures.

Bivariate regression, multiple regression, and factor analysis were used to determine if observed variation in six morphological characters of larvae (dependent variables) could be attributed to variation in various environmental factors (independent variables). The environmental variables considered are given in Table 9 and include monthly and annual long term average measurements of precipitation and temperature as well as altitude, latitude, longitude, and isophane. Significant relationships were found between various combinations of long term environmental variables and six larval characters, suggesting the presence of adapted gene complexes resulting from natural selection.

INTRODUCTION

This paper is a statistical study of the geographic variation and covariation of morphological characters of the rabbit tick, *Haemaphysalis leporispalustris* (Packard). Ticks from 122 localities distributed from Fairbanks, Alaska, south to San Diego County, California, southeast to Brownsville, Texas, and Broward County, Florida, and north to Fredericton, New Brunswick, were studied.

In a companion study (Thomas, 1967), the variation and covariation in larval, male, and female ticks were examined in some detail, as was the concordance of geographic variation of the three stages over 33 localities. All characters examined differed significantly among localities. Correlations of tick characters were computed as product-moment coefficients within localities and as product-moment and component coefficients among localities. Principal axes factor analysis with rotation to simple structure explained covariation in terms of fewer variables (factors). Six tick characters best representing interlocality factors will be utilized in the present paper for economy in description of geographic variation.

This study of geographic variation of *H. leporispalustris* was designed to achieve four main objectives: (1) Description of the geographic variation pattern of this widely distributed species. (2) Categorization of possible infraspecific units. (3) Demonstration of the use of statistical methods in geographic variation studies. (4) Quantification of the relationship of variation in larval ticks to putative causal variables.

Patterns of variation can give indications of the past distribution, rates and directions of gene flow, and nature of possible selective agents.

The biology of *H. leporispalustris* and its relations to variation and covariation of tick characters has been considered in some detail in Thomas (1965) and Thomas (1967).

MATERIALS AND METHODS

This study is based upon tick specimens from 122 localities distributed throughout North America north of Mexico. The main emphasis is on larvae because they were the most abundant life history stage and specimens were available from 120 localities. The analysis of variation of adult ticks was limited to localities from which larval samples were also available to allow comparison of patterns of variation of the three stages. Two additional localities having only adults were added to fill in gaps in the distribution of the adult stage. Table 1 is a list of the 122 localities used in this study. It contains the locality code number, locality descriptions, hosts, dates of collection and life history stages represented. If more than one sample was available for a given locality, this is noted and hosts and dates of the additional collections are given. Figure 1 is a map of North America showing distribution and code numbers of the various localities.

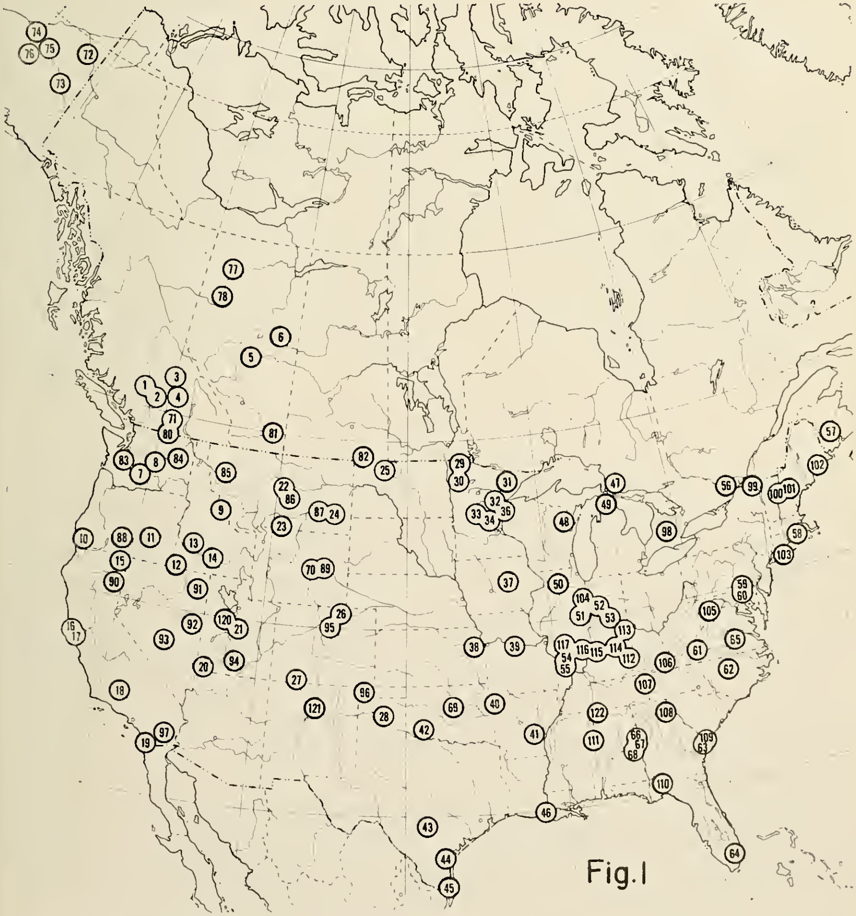

Fig. 1

FIGURE 1. Map of locality code numbers used in this study. A detailed listing of the localities is given in Table 1. Localities are represented by center of circles. Four of the 122 localities are not plotted: 118 and 119 lacked sufficient locality information and 79 and 35 were indistinguishable from 71 and 36, respectively.

The 16 tick characters studied and methods of preparation and measurement have been described in detail in the companion paper (Thomas, 1967).

Statistical computations were carried out on a desk calculator and on IBM 1620 and 7040 digital computers using programs written in FORTRAN II and IV.

DESCRIPTION AND ANALYSIS OF GEOGRAPHIC VARIATION

a. Character Variation

The analysis of tick variation was carried out as single classification analyses of variance (Steel and Torrie, 1960), one for each character. In general, there was a high degree of differentiation among localities for all characters. As pointed out by Sokal and Rinkel (1963), the analysis of variance in geographic variation studies can be interpreted as both a fixed treatment effect (Model I) and random effects yielding variance components (Model II) depending on the interpretation of the data. In Thomas (1967), the localities were considered as random samples from a large population of localities and variances for each character were partitioned into within and among locality components (Model II). In this study, a comparison of means from different localities is of interest and a Model I interpretation of the data is utilized. To obtain information regarding the geographic variation patterns, means and estimates of the average standard deviation were calculated for all characters at each locality in four studies described below.

64-Locality study of larvae

A preliminary study of 16 characters of larvae was made to obtain an idea of the overall pattern of character variation within and among localities. Since equal sample sizes simplify computations, only localities having a minimum of 15 ticks per locality were included in this analysis. At each locality the larvae were usually from a single host individual although samples of ticks from several host individuals were used at a few localities to obtain the required sample size of 15. The localities used in this study are designated as locality code numbers 1 through 64 in Table 1. The means (in microns) and an estimate of the average standard deviation, obtained by taking the square root of error mean square from the analysis of variance, are given in Table 2.

Factor analysis of the interlocality component matrix of 16 characters from 64 localities (Thomas, 1967) revealed three independent trends of variation (factors) which could be represented by only six characters. The two characters having the highest loading on each of the factors (the three independent trends of variation) were chosen for subsequent analyses. These characters are width of scutum and basis capituli, and length of genu III, tibia III, palp, and hypostome.

Means of 16 larval characters from the 64-locality study were plotted on maps and patterns of variation examined. The six characters chosen by factor analysis as representative of the three independent trends of variation adequately illustrated all trends of character variation observed. Further economy of description of patterns of geographic variation was obtained by considering the variation in terms of factors, as discussed below.

120-Locality study of larvae

To determine the effect of increasing the number of localities sampled on stability of geographic variation patterns in the six characters selected from the 64-locality study, larvae from 56 additional localities were included with the original 64 and the data reanalyzed. The additional localities, containing unequal sample sizes, are given in Table 1 as locality code numbers 65 to 119 and code number 122. In consideration of evidence presented in Thomas (1967), concerning variation of characters of ticks from different host individuals of the same species within a locality, ticks were randomly chosen from as many collections as were available at each locality to obtain a more representative sample. Table 3 lists the means, sample sizes, and estimates of the average standard deviations for the six larval characters. Although the first 64 localities are the same in the 64- and 120-locality studies, the means obtained are not necessarily identical because the ticks were sampled from all host individuals available at each locality in the 120-locality study, resulting in a different sample of tick specimens where more than one host individual was available.

38-Locality study of male ticks

An analysis of variation of six characters of males was made within and among 38 localities. The six characters used were homologous to those in the previous larval study and the following female study. The samples were variable in size, with a maximum of 15 ticks per locality, and were usually from more than one host individual. Localities represented by males are indicated in the right-hand portion of Table 1. Means, sample sizes and average standard deviations are given in Table 4.

36-Locality study of female ticks

Females from 36 localities were analyzed for intra- and interlocality variation. Sample size was variable with a maximum of 15 ticks from one or more host individuals from each locality. Localities represented by females are indicated in the right-hand portion of Table 1. Means, sample size and average standard deviations are given in Table 5.

b. Geographic Variation of Factors

Figures 2, 3, and 4 summarize geographic variation of the characters of

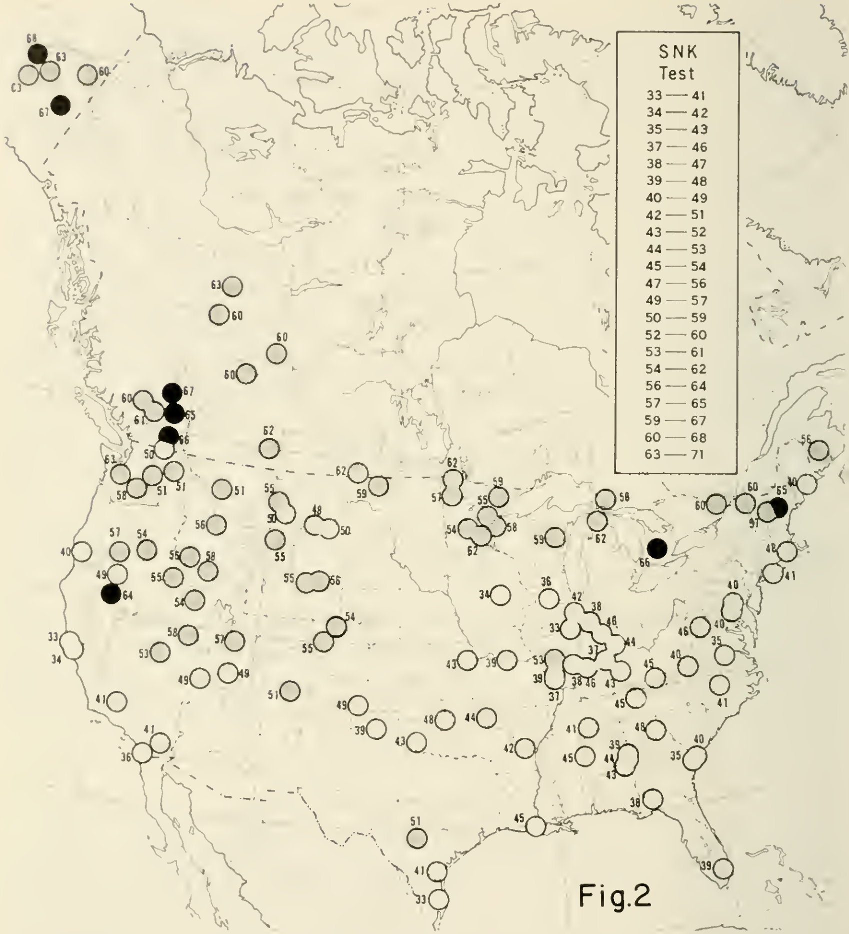

Fig.2

Figure 2. Geographic variation of interlocality factor I (body size factor). Each locality is represented by the mean of the standardized scores of the two characters most highly loaded on the factor. Shaded circles represent classes of standardized scores (10 times); white ≦ 36, light gray 37-50, dark gray 51-63, and black ≧ 64. The numbers next to the circles are the standard scores for that locality times 10. The results of the SNK test are given in the insert at the upper right of the figure. Any two standard scores included within ranges of values in any line of the SNK test results are not significantly different (at $P<0.01$).

larvae over 120 localities in terms of three factors. Each factor is equivalent to a suite of characters and is represented by the average scores of the two characters most highly loaded on that factor. The rationale behind this technique as well as the details of computation are given in Sokal and Rinkel (1963). Since the two characters used to represent each factor usually differed considerably in size, the scores had to be standardized before taking average values for each locality. Standardization was accomplished by

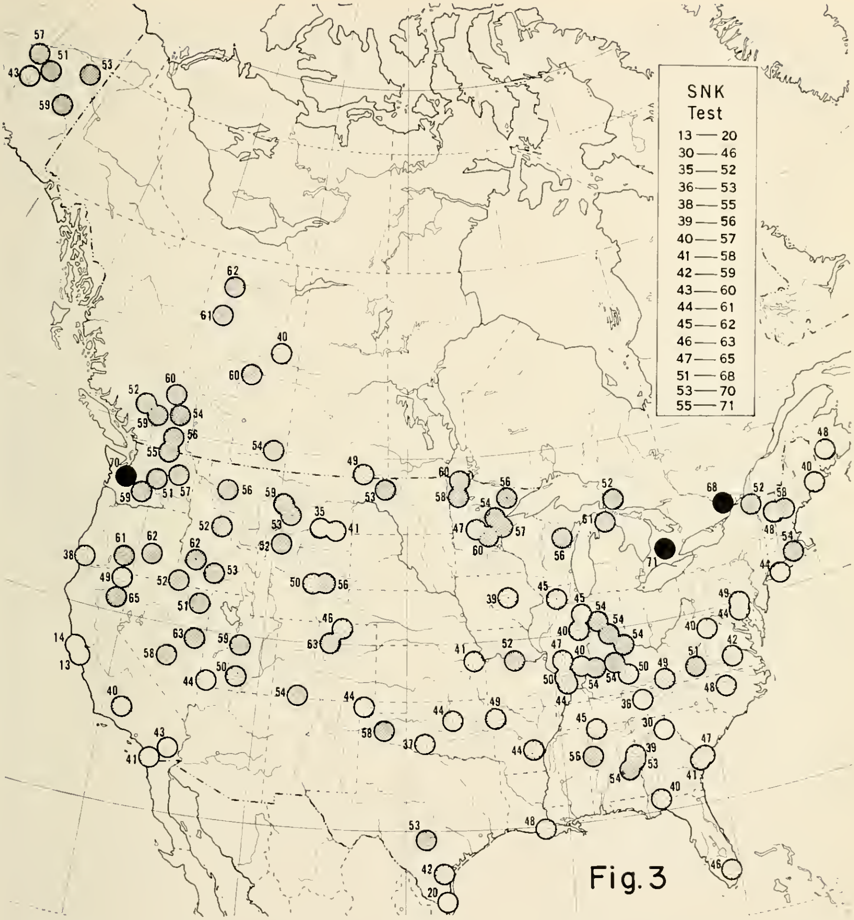

Fig. 3

Figure 3. Geographic variation of interlocality factor II (appendage factor). Explanation as in Figure 2.

dividing the difference of each locality mean from the grand mean of localities by the standard error of localities. The standard error of localities was obtained as the square root of the locality mean square divided by n_0. To avoid negative values, 5.0 was added to the standardized scores and to eliminate decimal points, the resulting scores were multiplied by 10. Therefore a locality with a mean identical to the grand mean has a score of 50. A locality score of 62 represents a mean 1.2 standard deviations greater than the grand mean. To obtain factor scores for each locality, the standardized means for the two characters representing the factor were added together and divided by 2. These values were plotted on Figures 2, 3 and 4.

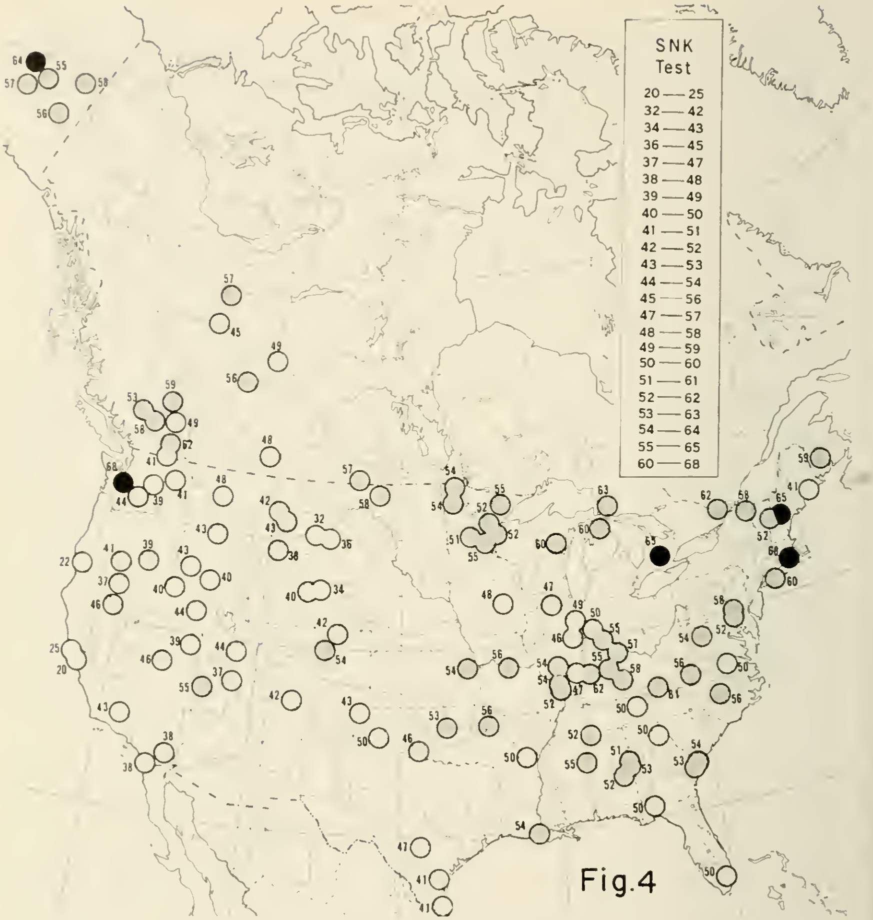

Fig.4

FIGURE 4. Geographic variation of interlocality factor III (capitular appendage factor). Explanation as in Figure 2.

The general organization of the maps is the same as in Sokal and Thomas (1965). Each map contains the standard scores coded as indicated above. Shaded circles represent classes of standard scores to emphasize patterns of variation. Values greater than 63 are colored black, those between 51 and 63 are dark gray, between 37 and 50 are light gray and those less than 37 are white. These classes are arranged so that in a normal distribution of standardized scores, with a variance of one, the extreme classes comprise 10% of the distribution at each tail and the two central classes make up 40% each. Since the correlation of the two variables representing each factor is not unity, the standard error of their sum is less than one and the number of locality scores expected in the extreme classes is less. For this reason the expected number of localities having scores in the extreme classes will be given separately for each of the factors upon consideration of the appropriate standard errors. These are 0.94 to 0.99 so that the deviations from expectation on the basis of unity are small. Circles of a diameter of 75 miles were used, instead of continuous shading between areas of equal magnitude, to avoid implying scores for unsampled areas.

Tests of significant differences between any pair of locality means are required. A number of graphic methods for comparison of several means have been developed (Hubbs and Perlmutter, 1942; Sokal, 1965). As pointed out by Pimentel (1959), Sokal and Rinkel (1963), and Sokal (1965), a multiple comparisons test is appropriate when a number of population means are to be compared. Tests of this type used in studies of geographic variation include Tukey's honestly significantly difference procedure by Ehrlich (1955), Duncan's multiple range test by Mason (1965) and the Student-Newman-Keuls (SNK) multiple comparisons tests by Sokal and Rinkel (1963) and Sokal and Thomas (1965). The latter method was applied to the means in this study. In Figures 2, 3, 4, and 5, any two standard scores included within ranges of values in any one line of the SNK test results are not statistically different at ($P < 0.01$). The procedure for calculating SNK values using unequal sample sizes has been discussed in Sokal and Thomas (1965). The q values used were obtained from tables in Harter (1960). An average sample size, n_0 (see explanation of Table 3 for formula), was used as an approximation for sample size. This method resulted in a 7% misclassification of significance of means in a study by Sokal and Thomas (1965). This loss of precision seems warranted in view of simplification of interpretation of the analysis as compared to a more exact method which considers sample size of each locality.

Figure 2 shows the pattern of variation of factor I, which might be called a body size factor and is represented by means of the width of scutum and basis capituli. There is a very pronounced north-south cline of mean values with values decreasing in a southward direction except in the west where high means occur southward in the Rocky Mountains. Some of the lowest means occur in Iowa and Illinois. The distribution of factor scores is symmetrical and somewhat bimodal (platykurtotic). The expected number of scores in the two extreme classes based on a normal distribution is 9.55. There is a very good agreement between this and the observed numbers, 9 white and 8 black circles.

Factor II (Figure 3) can be thought of as an appendage factor and is represented by the means of lengths of genu III and tibia III. This factor shows a less pronounced north-south cline with size decreasing southward. High means also occur in the Rocky Mountain area and are scattered throughout the central and eastern United States. Low values are sporadic in occurrence and are found in California, eastern Montana, southern Texas, eastern Tennessee, and northern Georgia. The distribution of factor scores is skewed to the left. There is a shortage of scores in the extreme classes, six white and four black, contrasted to the expected 11 each.

Factor III (Figure 4) is a capitular appendage factor represented by the means of lengths of hypostome and palp. There is a pronounced east-west cline with the values increasing eastward. In addition, high scores are found in the northwest of the continent. The distribution of standard scores is platykurtotic. There is a shortage of scores in both of the extreme classes with six white and five black localities observed contrasted to the expected 9.55.

In general the pattern established by the original 64 localities is confirmed and further elaborated by the additional 56 localities. There are several discrepancies such as the locality in Maine with low scores for all factors, but this is based on only two specimens.

An interlocality study of covariation of larval and adult characters showed that adults were similar to larvae in many respects but possessed an independent dimension of variation, factor IV, the "adult" factor (Table 18 in Thomas, 1967). Factor IV was represented geographically by averaging standardized scores of length of genu III and length of tibia III of the males for each locality and is mapped in Figure 5. Relatively large standard scores are found across the northern states and southern Canada and southward into Oregon, Idaho, Utah, Oklahoma and Texas. There is an excess of localities with scores near the mean locality value and a shortage of localities near the tails. The expected number is 3.5 but only one individual is in the low class and 2 are in the high class.

The SNK procedure showed that ticks from three localities in Figure 5 were significantly different from all others. Ticks from San Mateo County, California, were significantly smaller while ticks from Commanche County, Oklahoma, and Bexar County, Texas, were significantly larger. The southern Texas locality is of interest because two samples of ticks from this area were used in the analysis. One was a collection of ticks obtained May 29, 1955, from jackrabbits in Bexar County, Texas. The other sample was from a colony of ticks established from this collection and reared under laboratory conditions for eight years. Comparison of males and females of laboratory ticks and wild-caught ticks revealed significant differences in several characters. However, these ticks were similar in many other characters and even in those characters which showed significant differences the laboratory ticks were larger than any other locality considered in this study. The retention of large size after eight years under laboratory conditions is suggestive of a genetic basis for this character.

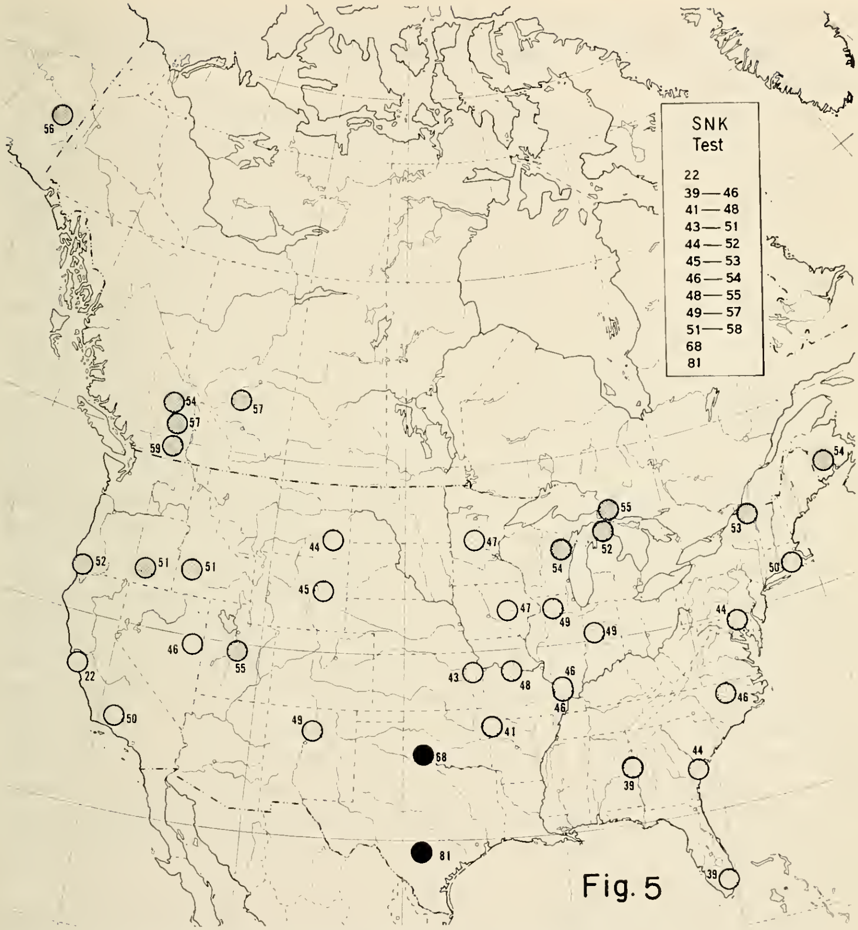

Figure 5. Geographic variation of interlocality factor IV (adult factor). Explanation as in Figure 2. This factor is based on male characters from 36 localities.

c. Projection of Localities in a Factor Space

A multidimensional space must be used to consider simultaneous variation of discordant characters, the phenetic position of a tick population (locality) in this hyperspace being determined by mean values of all characters considered. By calculating the first three principal axes of the character correlations and determining the projection of localities on them, optimal 1-, 2- and 3-dimensional views of the relative position of localities in the character hyperspace can be obtained. This combination of statistical and graphic methods has been used by Rohlf (1967) in enumerating relationships of OTU's in numerical taxonomic studies. This method is also similar to those used by DuPraw (1965) in his non-Linnean taxonomy of honeybees and by Jolicoeur (1959) in the description of multivariate geographical variation in the wolf.

The correlations of six tick variables chosen by previous factor analysis as representative of variation observed in 16 larval characters were extracted from the original interlocality component correlation matrix (Table 9 in Thomas, 1967) based on localities 1 to 64. This new 6 x 6 matrix was subjected to principal axis factor analysis using procedures described in Thomas (1967). The first factor extracted 70.46% of the total variation, providing the optimal one-dimensional view of the multidimensional pattern of variation. The second factor accounted for 17.37% of the variation and the third removed 10.65%. The total amount of variation explained was 98.50%, indicating essentially all of the covariation in the six characters was explained by three factors. Each factor was normalized by dividing its loadings by the square root of the eigenvalue of that factor, the eigenvalue being the sum of the squares of the factor loadings; this resulted in changing each factor to unit length. A matrix of character means for a number of tick populations (localities) was assembled and standardized by characters. This matrix had six columns corresponding to the number of characters and 74 rows corresponding to the number of localities available with a sample size of 15 larvae per locality. The standardized matrix of character means was post-multiplied by the normalized factor matrix having three columns corresponding to the three factors and six rows corresponding to the six characters. The product is a 74 by 3 matrix giving the coordinates of the 74 tick localities on the three principal axes (see Table 6). Each of the factors can be thought of as a linear combination of six larval characters from the correlation matrix. Although each factor affects mostly two of the characters, it does affect all six to some degree.

Since the first two factors account for 87.83% of the variation observed in six characters, a two-dimensional scatter diagram obtained by plotting coordinates of the 74 localities relative to the first and second factor axes gives an excellent two-dimensional representation of total variation. Figure 6 is such a two-dimensional scatter diagram showing the localities plotted against factor I (the long axis) and factor II (the short axis). The numbers next to the dots representing the localities refer to their code number. Inspection of

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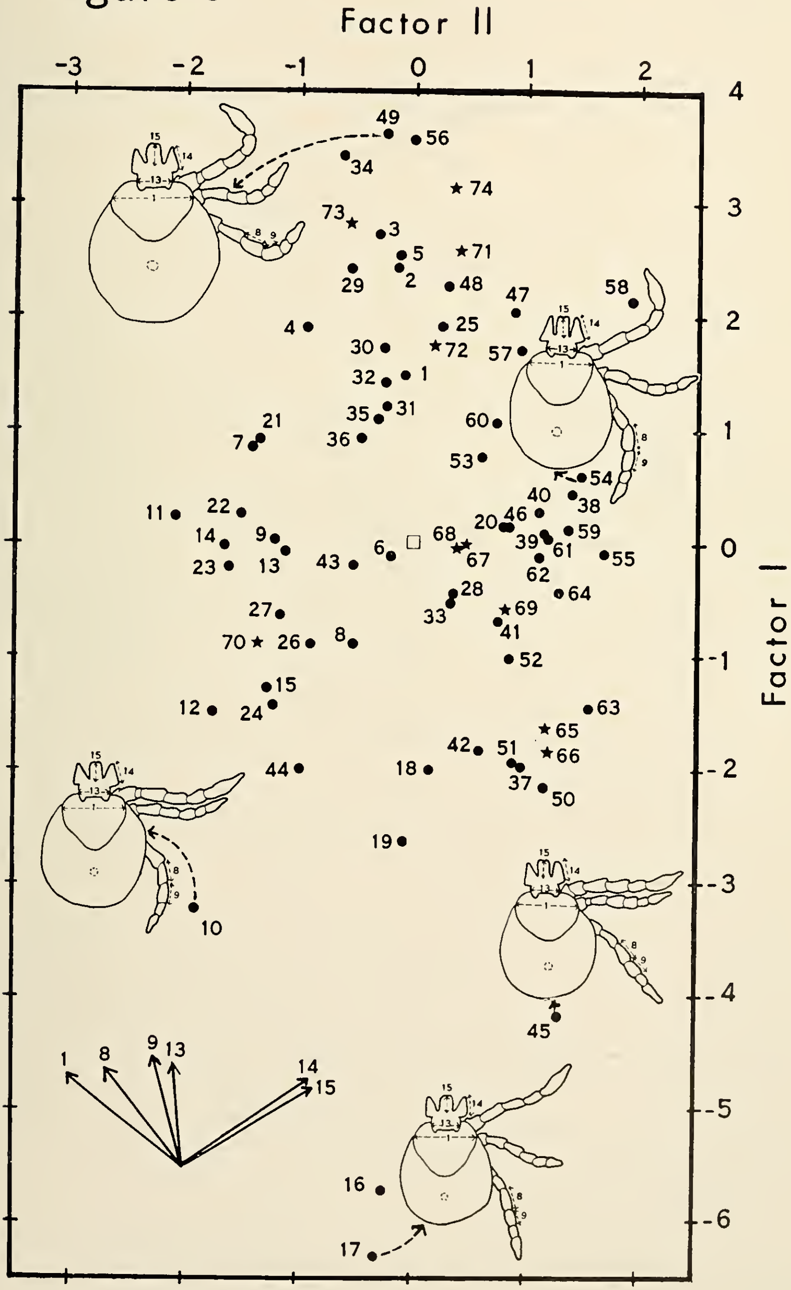
FIGURE 6. Scatter diagram of 74 localities plotted against principal axes I and II. A complete listing of coordinates is given in Table 6. Numbers next to dots and stars refer to locality code numbers given in Table 1. Localities indicated by black dots are used in calculation of principal axes. Localities represented by stars were not used in the original factor analysis and were included to test predictive ability of the method for "unknown" specimens. Arrows represent vectors of characters obtained by plotting their normalized principal component loadings on factor I against factor II. The small square within the scatter diagram represents the point 0, 0. The drawings of larval ticks are tracings of representative specimens from the populations indicated by dashed arrows. The numbers and dashed lines with arrows on the ticks refer to the code numbers of the six characters used in this analysis.

Figure 6


Factor II
-3
-2
-1
0
1
2
Factor I
4
3
2
1
0
-1
-2
-3
-4
-5
-6
49
56
34
74
73
3
71
5
29
2
48
47
58
4
25
30
72
57
32
1
35
31
21
7
36
60
53
54
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11
22
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65
42
51
66
44
18
37
50
19
10
45
16
17
1
8
9
13
14
15

dispersion of localities relative to the two factor axes shows a remarkable correspondence to geographic distribution. In fact, the long axis can be considered to represent a north-south direction with north being towards the top of the page and the short axis an east-west direction with east being to the right of the page. Localities plotted in the upper right hand portion of the graph generally have a northeastern geographic origin, likewise localities in the lower left portion of the graph are southwestern or western in origin.

The arrows in the graph represent vectors of the characters and illustrate the direction of variation of characters with respect to the factor axes. The vectors were determined by plotting factor loadings of the characters on factor I against factor II. The length of the vector is equal to the square root of the communality and direction of the arrow indicates direction of increase of a given character. The drawings of larval ticks included in the diagram are tracings of representative ticks from populations indicated and illustrate the changes in proportions and size of characters in relation to these two factor axes. Consideration of the character means given in Table 3 is also helpful in interpretating these changes. Ticks from locality 10 (—1.9, —3.2) are similar to those from locality 54 (1.5, 0.6) in size of scutum and genu (characters 1 and 8). This similarity can be predicted by considering the direction of the character vectors and the relative position of the two localities. The two localities lie on a line perpendicular to character vectors 1 and 8. Locality 17 (—0.3, —6.3) differs from locality 10 (—1.9, —3.2) in width of scutum, length of genu III, length of tibia III and width of basis capituli. This variation again can be predicted by considering the direction of character vectors related to the direction and distance between two localities. Larvae from locality 17 (—0.3, —6.3) and locality 49 (—0.2, 3.6) are nearly identical in their loadings on the second axis so they are similar in proportions but ticks from the latter locality are much larger because all of the character vectors show an increase in the positive direction.

The stars represent localities not used in the original factor analysis or "unknowns" according to DuPraw's (1965) terminology. By using the principal axes based on the 64-locality study these additional 10 localities were properly placed according to their geographic relationships to the other localities. For example, localities 74 and 71 occurring at the top of the graph are ticks of northern origin, from Alaska and British Columbia, respectively. Localities 66 and 65 at the lower right hand of the graph are localities from Alabama and Virginia. Locality 70 in the left center of the graph is from Wyoming.

A consideration of the pattern of points shows that there are three main clusters centered in the north, east, and west with the possibility of a fourth group from the west and southwest represented by localities 16, 17, 19, 10 and 45. The center of gravity of the cluster of points, indicated by a hollow

Figure 7

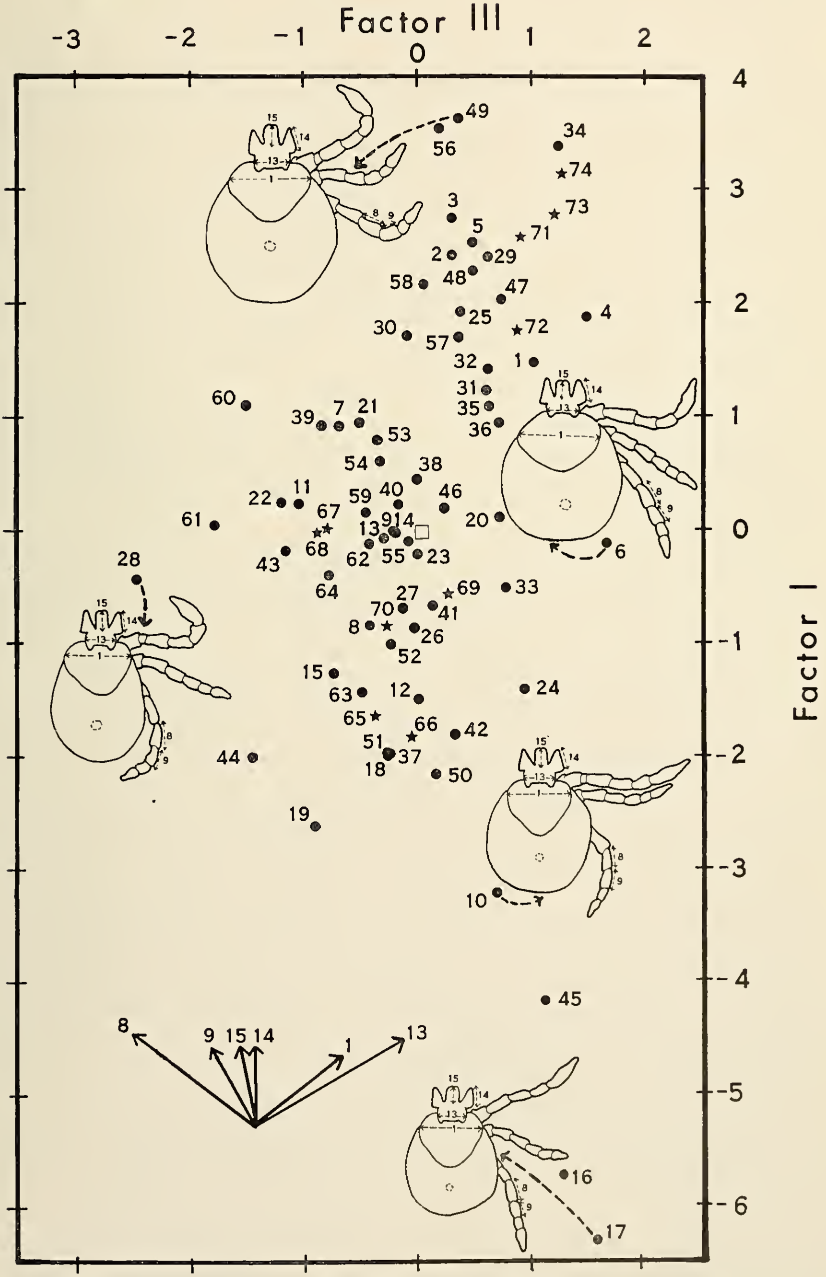

Figure 7. Scatter diagram of 74 localities plotted against principal axes I and III. Explanation as in Figure 6.

square, is at the intersection of the two factor axes (0, 0) owing to the standardization of characters.

The clusters of localities appearing to be closely related in this scatter diagram may be separated when a third dimension is considered. Figures 7 and 8 show the other two possible combinations of the three principal axes. The character gradients influenced by factor III are not oriented in north-south or east-west directions so clusters of localities in two-dimensional graphs containing this factor will be positioned relative to geographic origin in one dimension (factor I or factor II) only. The ability of a third dimension to differentiate localities closely related in two dimensions is well illustrated by comparing Figures 7 and 8. In Figure 7, localities 18, 37 and 51 (at —0.5, —2.0) have essentially the same coordinates on factors I and III. When considered relative to factor II the three localities are shown to be distinct (see Figure 8).

All characters have positive loadings on factor I so variation in this dimension represents size differences. Factor II and III have both positive and negative loadings, so character variation in these dimensions represents changes in proportions.

In Figure 7 there is a north-south dispersion of localities relative to factor I, but the east and west clusters of localities of Figure 6 are interspersed, indicating considerable variability in character proportions represented by factor III.

In Figure 8 there is an east-west orientation of localities on factor II but the north-south orientation is less distinct. Inspection of character vectors in Figure 8 show that ticks with high positive scores on factor III have both a proportionately wider basis capituli (character 13) and shorter genu and tibia III (characters 8 and 9). Ticks with high positive loadings on factor II have proportionately longer palps and hypostome (characters 14 and 15) and a proportionately narrower scutum (character 1). The two dimensions in this figure are proportion factors so ticks having similar shapes now cluster together irrespective of size differences. This is illustrated by the closeness of localities 16 and 17, which have very small ticks, to localities 6, 4, 34 and 73, which have very large ticks. A consideration of the relationships of tick populations with respect to these two factor axes thus eliminates the need for ratios to remove size effects.

To visualize the relative position of each locality in all three dimensions simultaneously, a three-dimensional model was constructed using styrofoam balls and wire. The distance between the localities is a measure of their phenetic similarity, i.e., the closer two localities are the more similar are the ticks from the two localities. Figures 9, 10 and 11 are photographs of this three-dimensional model. The long axis of the styrofoam base represents factor I, the short axis represents factor II and the wires perpendicular to

Figure 8

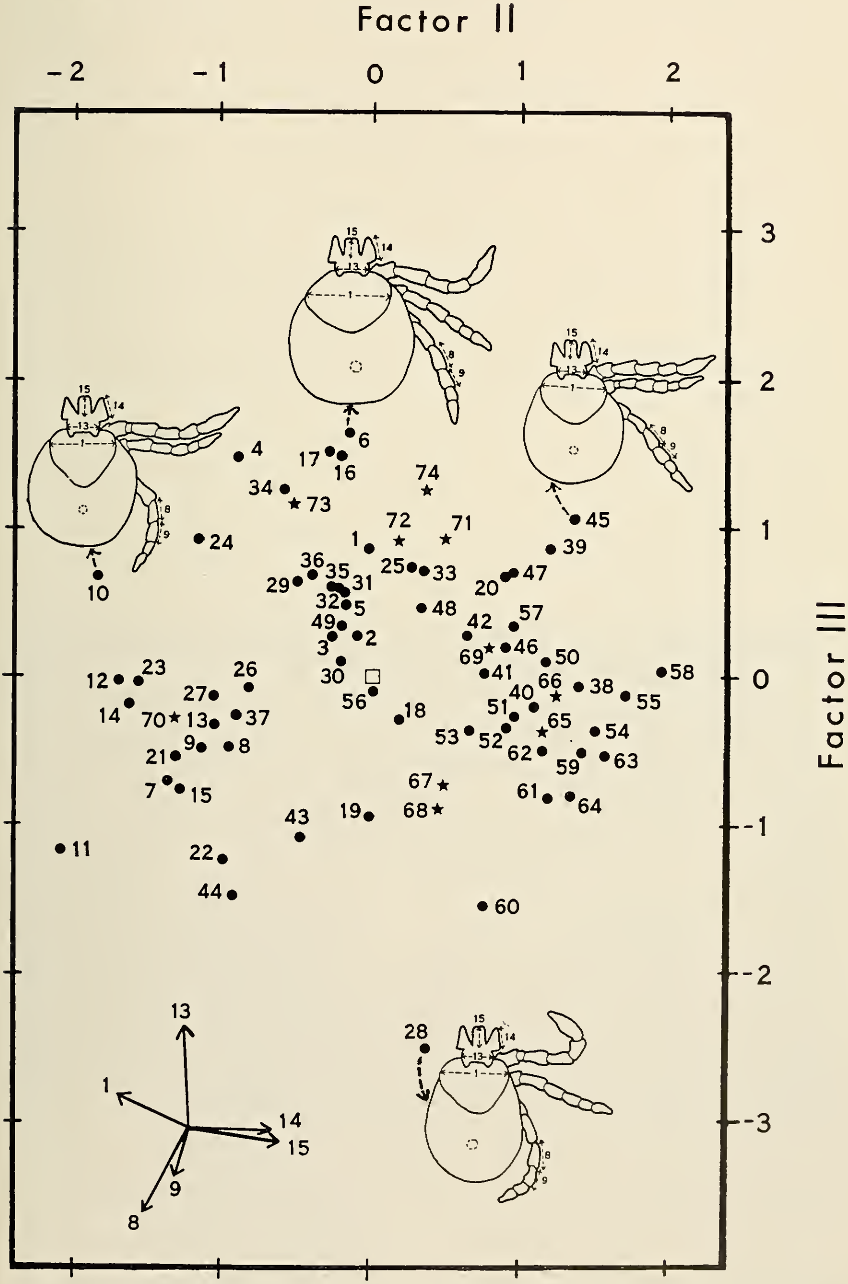

Figure 8. Scatter diagram of 74 localities plotted against principal axes II and III. Explanation as in Figure 6.

the base represent factor III. The styrofoam balls with numbers represent localities and the styrofoam ball with a number of small spheres radiating from it represents the character vectors in three dimensions and is positioned at —5, 0,0 on axes I, II and III. Placing it at 0,0,0 would have been awkward since this is the center of gravity of the cluster of points. The long and short axes of the styrofoam base represent north-south and east-west axes of the two-dimensional scatter diagram (Fig. 6), respectively.

Three photographs of the three-dimensional model illustrate how different interpretations of clusters of localities can be made by considering the model from various aspects. Figure 9 is a view from a position positive on factor axis II and zero on axes I and III emphasizing the variation of localities in the third dimension (factor III). The northern group is characterized by high loadings on this factor while the eastern and western groups, which are not distinguishable from this view, are quite variable in this dimension. The variability observed in this dimension can be explained in part by considering the character vectors. Characters 8 and 9 and 1 and 13 can be seen to have opposite effects in that the vectors of 8 and 9 point downward and vectors of 1 and 13 point upward. Therefore the third dimension represents the resultant of interaction of these four characters. Again factor I represents general size differences whereas factors II and III emphasize differences in proportion of various characters.

Figure 10 is another aspect emphasizing the third dimension. It is taken from a position negative for factors I and III and positive on factor II and shows the division of the east and west groups not apparent in Figure 9. From this view the northern group appears to be part of the eastern cluster.

Figure 11 is a view looking down at the model from a position negative for factors I and II and positive on factor III. This view shows the optimal differentiation of localities into four distinct clusters. The clusters of localities produced do not appear to be an artifact of the sampling of localities because localities in the outer portions of the clusters do not necessarily correspond to the edges of the distribution for the localities in the group, i.e., the gaps between the various clusters are not produced by gaps in the geographical distribution between these localities. If this were the case, additional samples of localities from missing areas would tend to fill in the gaps between localities and the various clusters would lose their distinctness.

It is tempting to create infraspecific groups based on the dense clusters of localities having similar geographical relationships and a minimum of polytopy. However, such an attempt to create arbitrary groupings is subject to the same criticisms that DuPraw (1965) has made concerning the assignment of species to Linnean categories. Categorization leads to information loss by failing to emphasize the high relative similarity of specimens to either side of a category boundary, the dissimilarity of extreme specimens within a category, and the degree of similarity between specimens in one category relative to specimens within another.

PUTATIVE CAUSES OF DIFFERENCES IN LARVAL CHARACTERS

The computations discussed in the previous section showed clinal variation in several factors representing suites of correlated larval characters. There was a north-south cline for the body size factor and an east-west cline in the capitular appendage factor. Since local populations are under continuous selection pressure to adapt to conditions of the particular area in which they occur, clinal patterns of variation should reflect corresponding gradual changes in selective forces. Climate is often assumed to be the selective force responsible for gradual character changes because it varies slowly and regularly over wide areas, except along coasts and in mountains.

a. Regression Analysis.

Regression analysis can be used as an explanatory device to determine if a portion of the observed variation in morphological characters (dependent

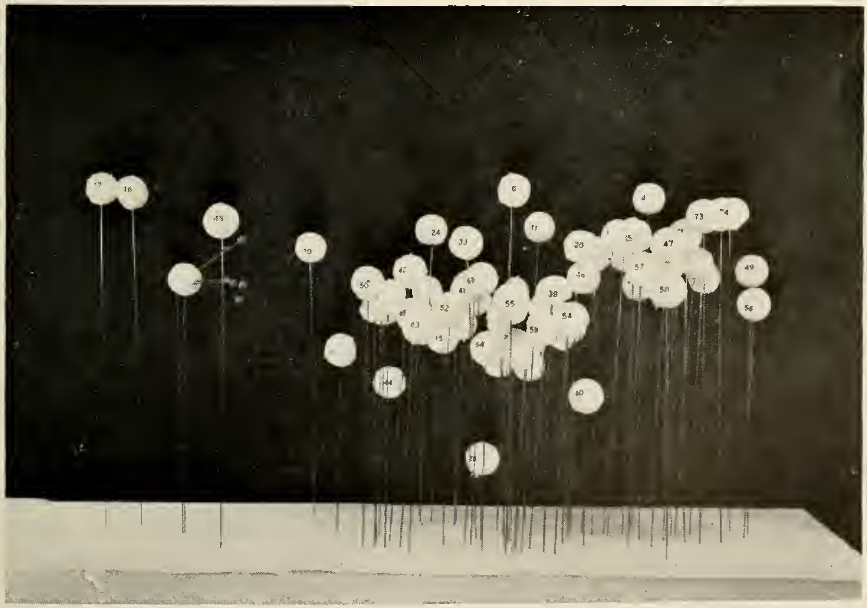

Figure 9. Three-dimensional model of localities plotted relative to axes I, II and III. View from position positive on factor axis II and zero on factors I and III. Long axis of base represents factor I, short axis of base represents factor II and wires represent factor III. Numbered styrofoam balls represent localities listed in Table 1. Unnumbered styrofoam ball and small spheres radiating from it represent 0 on factor III and character vectors in three dimensions, respectively.

variables) can be attributed to variation in climatic factors (independent variables). Significant regression of a character on an environmental variable does not distinguish whether the relationship is based on the direct influence of the environment or on a genetically based adaptation to past action of the environmental variable or on a combination of these two phenomena.

To elucidate possible causes of the observed variation pattern in *H. leporispalustris*, six larval characters were regressed on a number of environmental variables. The independent variables were weather data obtained from published records of the U.S. Weather Bureau, altitude, longitude, latitude, and isophane of the localities. The isophane is a statistic developed by Hopkins (1938) reflecting gross climatic features, especially temperature. The formula for the isophane is as follows: position isophane in °N= (position latitude in °N) + 1/5 (100 — position longitude in °W) + (altitude in feet/400). Sixty-two localities from the United States having a sample size of 15 ticks for each locality were selected.

Weather records were obtained from the Climatic Summary of the United States—Supplement for 1931 through 1952 published for each state and available from the Superintendent of Documents, U.S. Government Printing Office, Washington 25, D.C., and from annual summaries of climatological data published for each state from 1953 to 1964 and available from the same

FIGURE 10. Three-dimensional model of localities plotted relative axes I, II and III. View from position positive for factor axis II and negative for factor axes I and III. Explanation as in Figure 9.

source as the previous weather records. The microenvironment experienced by the ticks undoubtedly differs from the climatic data in these published records, but the latter are representative of general conditions existing in a given locality.

The weather data used as independent variables in this analysis included long term annual means of total precipitation, temperature, maximum temperature, and minimum temperature. Long term monthly mean values for one and two months previous to the date of collection of the sample of ticks, and the month of collection were recorded for each of the previously mentioned weather variables. Total precipitation for the month of collection of the tick samples as well as for one and two months previous and for the entire year were also recorded. Weather records from one and two months previous to the date of collection were introduced to determine possible lag effects of weather conditions on tick characters. Conditions prevailing during time of engorgement of the female, during egg development or when the larvae are newly hatched and unable to attach to a host may have an important influence on characters of larvae. The two month lag was chosen because larvae from a host are at least two months old, including developmental time of eggs. A similar approach was made by Hazelwood and Parker (1963) in studying the lag effect of certain environmental factors upon population size of two zooplankters, *Diaptomus* sp. and *Daphnia* sp., by means of multiple regression techniques.

Bivariate regression of six larval characters (width of scutum, length of genu III, length of tibia III, width of basis capituli, length of palp, and length of hypostome) were calculated on the environmental variables previously discussed. All of the independent variables, except one and two month lags of long term mean maximum temperature and one month lag of long term mean temperature, affected at least one larval character significantly. The larval characters with significant regressions on total precipitation in the year of collection showed the same patterns on the long term averages for precipitation. In fact the relationships to long term mean total precipitation for two months previous, one month previous and month of the collection as well as annual mean total precipitation were much more pronounced than to precipitation from the year of collection. The stronger relationship to long term mean values seems to reflect adaptations to conditions of long term duration rather than direct influence of these variables on tick characters.

The relationship of environmental variables, considered simultaneously to tick characters was investigated by a multiple regression analysis. Owing to the similarity of effects of long term precipitation values to those of the year and months of the tick collections, the latter were eliminated in this analysis. The covariation of the remaining 20 independent variables and six larval characters was analyzed with the stepwise multiple regression pro-

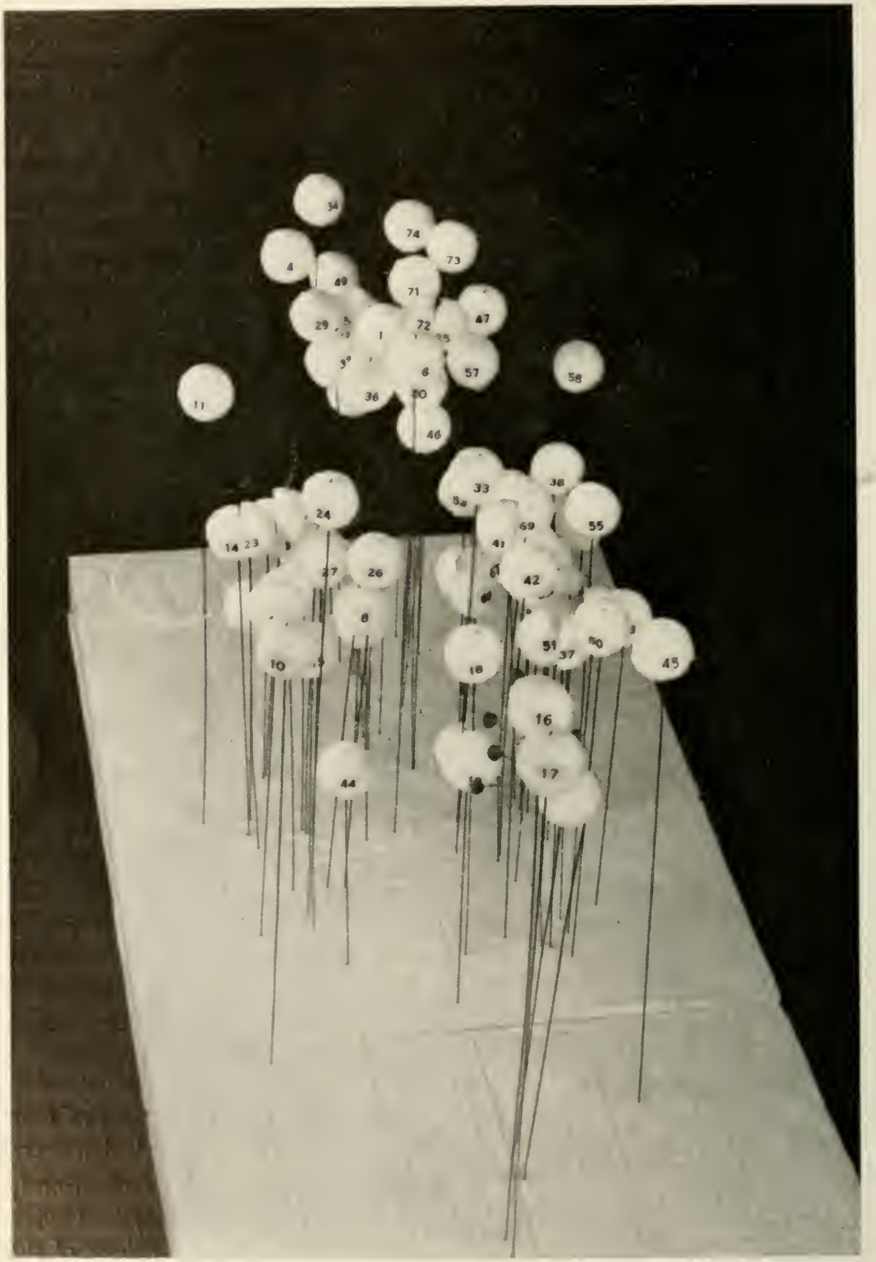

FIGURE 11. Three-dimensional model of localities plotted relative to axes I, II and III. View from position negative for factors I and II and positive on factor III. Explanation as in Figure 9.

gram developed by Efroymson (1960). His paper gives a detailed description and flow-chart of the program. The object of this program is to build up the best possible multiple regression equation for predicting a dependent variable from the independent variables considered. Independent variables are added to the equation, one at a time, with the criterion for selection being improvement in "goodness of fit" of the predicted to observed values. An important property of this method is that a variable indicated to be significant at an early stage and entered into the equation may become insignificant when additional variables are added. Insignificant variables are removed from the equation so only significant variables are included in the final regression. It is important to emphasize that different patterns of regression relationships would be illustrated if the dependent variables were regressed individually on these variables.

Table 7 lists the 20 independent variables considered and the six tick characters. For each tick character, significant ($P < 0.05$) partial regression coefficients, Y-intercept, multiple correlation coefficient and percentage of multiple determination, are given. The multiple correlation coefficient (R) represents the correlation of values predicted from the regression equation with observed values. The R values are relatively large in magnitude ranging from 0.68 for length of hypostome to 0.84 for width of basis capituli and length of palp. The percentage of determination, computed as $100 \times R^2$, is the percentage of variation of the dependent variable attributable to combined effects of the independent variables. It varies from 45.67% for length of hypostome to 70.62% for length of palp. Length of hypostome is relatively poorly explained by the independent variables considered. This structure is very important in attachment of the tick to the host and may be influenced by more direct selective factors not considered in this analysis, such as thickness of the host's skin.

The number of independent variables having significant partial regression coefficients in the different dependent variable regression equations varies from two for length of tibia III to six for length of hypostome. The most prevalent independent variables in the regression equations are various measurements of total precipitation, annual mean temperature, elevation, and isophane. The nature of the isophane term can be readily explained by inspection of the correlation of independent variables shown in Table 8. Isophane has a relatively small negative correlation with long term mean total precipitation and very high negative correlations with long term annual mean temperature, annual mean maximum temperature, and annual mean minimum temperature, and a positive correlation with latitude.

Estimated values for the dependent variables and the deviations of expected values from observed values were calculated for each character and locality using the regression equation. The deviations of the various characters were divided by their standard error to give a standardized score. These standardized scores were plotted on maps and inspected for possible patterns. A similar method was employed by Huntington (1952) in a study of purple grackles. Twenty-six of the 62 localities used in this analysis did not show appreciable deviations in any of the six characters from the predicted value. Five localities had four characters much smaller than predicted. These were Marin and San Mateo Counties, California, Tama County, Iowa, and Lee and Champaign Counties, Illinois. Four characters were much larger than predicted in Lee County, Alabama, and Cheboygan County, Michigan. All six larval characters were much larger than predicted in Mille Lacs County, Minnesota. The localities with poor character prediction were distributed throughout the range of the tick and no definite pattern could be established.

b. Factor Analysis.

Another approach to clarification of the interrelationships of environmental and morphological variables is through factor analysis. Cattell (1952) suggested that in a simple structure solution of a correlation matrix containing both response and suspected causal variables, any putative causal variables highly loaded on a given factor may actually be the factor itself. This approach was applied by Sokal, Daly, and Rohlf (1961) and Sokal and Daly (1961) to six physical and 19 biological variables with partial success.

The correlation matrix of 20 independent variables and six larval characters (Table 8) was factor analyzed by techniques described in Thomas (1967). Table 9 shows the primary pattern matrix of the six factors which resulted and the correlation among factors. Four of the six factors have loadings on both environmental variables and larval characters and therefore are of interest with regard to possible reification of factors. If the independent variables actually represent the factors appearing in this analysis they should have very high loadings on the factors. However, the dependent variables in factors V and VI have higher loadings than the supposed independent variables. This may indicate the presence of another causal nexus responsible for correlation of the independent and dependent variables. Nevertheless, inspection of the loadings on the various factors gives important clues to interrelationships of the variables and allows for prediction of clines in larval characters that are substantiated by inspecting maps of character variation given in Figures 2, 3, and 4.

Factor I shows the negative relationship between longitude and total precipitation. This factor also mildly affects width of scutum negatively and length of palp positively. Factors II and IV influence only physical characters and illustrate the positive effect of mean monthly temperatures on temperatures of the following month. Factor III illustrates the negative relationship of annual temperatures with latitude, longitude and isophane.

The width of basis capituli and scutum also show moderate loadings on this factor. Factors V and VI have high loadings on many tick characters as well as environmental variables and provide the best examples for predicting patterns of variation of tick characters. The high positive loadings by length of palp and length of hypostome and the negative loadings of altitude and longitude on factor V suggest that ticks should have smaller palps and hypostome in the western part of the range. This is indeed the case. The close relationship of isophane to latitude as well as their negative relationship to temperature is emphasized in factor VI. In addition the clinal variation of width of scutum and basis capituli, length of genu III and tibia III is indicated by this factor. The positive relationship shown between the tick characters and latitude suggests that ticks should be larger the higher the latitude which is the pattern of variation observed (see Figures 2 and 3).

The multiple regression equation and factor analysis provide quantification of the interrelationships between independent variables and characters of ticks. In many respects the factor analysis solution gives a clearer picture because it shows relationships of dependent variables to all independent variables regardless of their relative predictive ability. For this reason factor analysis gives a better overall impression of the various interrelationships, allowing for more meaningful interpretations.

Consideration of factor loadings shows that overall size of larvae is greatest in areas having low mean annual temperatures and total precipitation. The lengths of leg and capitular appendages are relatively greater than other body measurements in areas having high total precipitation during the season of larval abundance. The ticks in colder areas hibernate. For successful survival, greater amounts of body fat must be stored and hence a larger size may be favored. In drier areas smaller appendages may be favorable to maintenance to water balance by reducing surface area, possibly explaining the relationship of appendage length to precipitation.

Significant regression of morphological on environmental variables is not proof of the causal nature of the latter, but is suggestive of such a connection and should be further investigated. Since the environmental variables in this study represent long-term annual mean values, the apparent relationships between larval characters and environmental factors probably reflect adapted gene complexes resulting from natural selection.

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TABLE 1. List of Localities Employed in this Study.

Locality code number	Locality, host and date	Life history stage
1	BRITISH COLUMBIA, Marble Canyon on Fraser R. N of Lillooet; snowshoe hare; 25-VIII-1933.	L
2	BRITISH COLUMBIA, Deadman Creek, W end of Kamloops Lake; grouse; 14-VIII-1934.	L
3	BRITISH COLUMBIA, Vavenby, 66 m. NNE Kamloops; 3 samples from snowshoe hares; 14-VI-1931, 2-V-1932.	L, ♂, ♀
4	BRITISH COLUMBIA, Salmon Arm, 45 m. E Kamloops; snowshoe hare; 15-V-1931.	L, ♂, ♀
5	ALBERTA, Wabamun, 41 m. W Edmonton; *Lepus americanus;* 30-VIII-1963.	L
6	ALBERTA, Lac la Biche, 110 m. NE Edmonton; *L. americanus;* 17-VIII-1950.	L
7	WASHINGTON, Yakima Co., 10 m. NE Yakima; *Microtus longicaudus;* V-1947.	L
8	WASHINGTON, Grant Co., 11 m. S Moses Lake; 2 samples from *Sylvilagus nuttallii;* 29-VIII-1940.	L
9	MONTANA, Ravalli Co.; 4 samples from cottontails; 21-VI-1949, 15-IX-1931, 2-XI-1932.	L
10	OREGON, Josephine Co., Grant's Pass; jackrabbit; larvae: 4-VIII-1935; adults: 17-VI-1935.	L, ♂, ♀
11	OREGON, Harney Co., Burns; 3 samples from pigmy rabbits; 11-V-1933, 29-VIII-1932; 3 samples from cottontails; 19-VII-1932; 25-VII-1932.	L, ♂, ♀
12	IDAHO, Owyhee Co., Grandview; 2 samples from jackrabbits; 13-VII-1932, 15-VIII-1932; one sample from cottontail; 2-VI-1961.	L
13	IDAHO, Elmore Co., Mayfield Reservoir; 15 samples from jackrabbits; June to September 1932.	L, ♂, ♀
14	IDAHO, Lincoln Co., Shoeshone Falls; cottontail; 22-VI-1938.	L
15	CALIFORNIA, Modoc Co., 21 m. N Alturas; cottontail; 9-V-1949.	L
16	CALIFORNIA, Marin Co.; 2 samples from *Sylvilagus bachmani;* 26-VIII-1944, 2-IX-1944.	L
17	CALIFORNIA, San Mateo Co., larvae: spotted towhee; 22-VIII-1961; adults: cottontail; 23-VI-1961.	L, ♂, ♀
18	CALIFORNIA, Kern Co., 1 m. N Lerdo Road; *Sylvilagus audubonii;* 19-VII-1962.	L, ♂, ♀
19	CALIFORNIA, San Diego Co., Vista; cottontail; 8-II-1937.	L
20	UTAH, Washington Co., St. George; *Sylvilagus audubonii;* 4-VI-1961.	L
21	UTAH, San Pete Co.; chipmunk; 27-VIII-1936.	L
22	MONTANA, Fergus Co., Roy; cottontail; 23-IX-1931.	L
23	MONTANA, Carbon Co., Edgar; cottontail; 29-VIII-1930.	L
24	MONTANA, Custer Co., Miles City; cottontails; 10-VII-1930.	L, ♂, ♀
25	NORTH DAKOTA, Ward Co., Kenmare; *Lepus americanus;* 13-IX-1941.	L
26	COLORADO, Weld Co., Chalk Bluffs; *Sylvilagus* sp.; 22-VIII-1943.	L
27	COLORADO, Conejos Co., Antonito; 4 samples from cottontails; 9-VIII-1931, 12-VIII-1931, 13-VIII-1931, 14-VIII-1931.	L
28	TEXAS, Armstrong Co.; black-tailed jackrabbit; 6-VI-1963.	L
29	MINNESOTA, Roseau Co.; *Bonasa umbellus;* 2-X-1932.	L
30	MINNESOTA, Pennington Co., Thief River Falls; *Pediocetes phasianellus;* 24-VIII-1932.	L
31	MINNESOTA, St. Louis Co.; 2 samples from *Bonasa umbellus;* 3-X-1932.	L
32	MINNESOTA, Aitkin Co.; 2 samples from *Bonasa umbellus;* 30-IX-1932.	L
33	MINNESOTA, Morrison Co., Lake Alexander; larvae: 6 samples from *Lepus americanus;* 4 samples from *Sylvilagus floridanus;* and 4 samples from *Bonasa umbellus;* June to October 1932; adults: *Lepus americanus;* 21-IV-1932.	L, ♂, ♀
34	MINNESOTA, Mille Lacs Co., Wahkon; 2 samples from *Bonasa umbellus;* 23-IX-1932.	L
35	MINNESOTA, Kanabec Co., Mora; 2 samples from *Bonasa umbellus;* 23-IX-1932.	L
36	MINNESOTA, Pine Co.; 2 samples from *Bonasa umbellus;* 27-IX-1932.	L
37	IOWA, Tama Co., Tama Indian Reservation; 8 samples from cottontails; May to Sept. 1941.	L, ♂, ♀
38	KANSAS, Douglas Co., Lawrence; 7 samples from *Sylvilagus floridanus;* 16-III-1963, 28-VI-1963.	L, ♂, ♀

TABLE 1. List of Localities Employed in this Study (Continued).

Locality code number	Locality, host and date	Life history stage
39	MISSOURI, Boone Co., Columbia; cottontail; 9-V-1958.	L, ♂, ♀
40	ARKANSAS, Washington Co., Prairie Grove; 15 samples from cottontails; Oct. 1958, Mar., Apr., June to Sept. 1959.	L, ♂, ♀
41	ARKANSAS, Jefferson Co., Pine Bluff; 2 samples from *Sylvilagus aquaticus;* 4-XI-1954.	L
42	OKLAHOMA, Comanche Co., Wichita Wildlife Refuge; jackrabbit; 5-VIII-1942.	L, ♂, ♀
43	TEXAS, Bexar Co., Camp Bullis; adults: jackrabbit; 29-V-1955; adults and larvae: laboratory colony; 1963.	L, ♂, ♀
44	TEXAS, Kleberg Co., Kingsville; 2 samples from jackrabbits; 23-V-1938, 1-XII-1938.	L
45	TEXAS, Cameron Co.; meadowlark; 27-II-1951; cardinal; 31-I-1962; curved-bill thrasher; 11-I-1963; robin; 16-I-1963.	L
46	LOUISIANA, Iberia Co., Jeanerette; rabbit; 1930.	L
47	ONTARIO, Algoma District, Searchmont; *Lepus americanus;* 18-VI-1943.	L, ♂, ♀
48	WISCONSIN, Oconto Co., Lakewood; snowshoe hare; 23-VI-1947.	L, ♂, ♀
49	MICHIGAN, Cheboygan Co., Douglas Lake; 3 samples from *Lepus americanus;* 13-VII-1959, 12-VII-1961, 3-VII-1964; 2 samples from *Bonasa umbellus;* 2-VII-1961, 10-VII-1964.	L, ♂, ♀
50	ILLINOIS, Lee Co.; 6 samples from cottontails; August and September 1952, 1953.	L, ♂, ♀
51	ILLINOIS, Champaign Co., Urbana; cottontail; 4-IX-1947.	L
52	INDIANA, Tippecanoe Co., Lafayette; 2 samples from cottontails; 10-V-1958, 27-VII-1960.	L, ♂, ♀
53	INDIANA, Marion Co., Indianapolis; cottontail; 25-IV-1958.	L
54	ILLINOIS, Jackson Co., Carbondale; 8 samples from cottontails; July to Sept. 1956.	L, ♂, ♀
55	ILLINOIS, Union Co.; 7 samples from cottontails; July and August 1956.	L, ♂, ♀
56	ONTARIO, Carleton Co., Burritt's Rapids; crow; 16-VI-1963.	L
57	NEW BRUNSWICK, York Co., Fredericton; adults and larvae: *Lepus americanus;* 30-V-1957; larvae: ruffed grouse; 27-IX-1951.	L, ♂, ♀
58	RHODE ISLAND, Washington Co., Kingstown; 5 samples from cottontails; 23-VII-1956, 11-IV-1957, 25-VI-1957, 24-VII-1957.	L, ♂, ♀
59	PENNSYLVANIA, York Co., Jacobus; 2 samples from *Sylvilagus floridanus;* 29-X-1955, 1-XI-1955.	L
60	MARYLAND, Anne Arundel Co., Patuxent Wildlife Refuge; larvae and adults: 3 samples from cottontails; 28-IV-1944, 26-III-1954; larvae: 7 samples from bobwhites; 13-X-1956.	L, ♂, ♀
61	VIRGINIA, Montgomery Co., Blacksburg; bobwhite; 20-XI-1945.	L
62	NORTH CAROLINA, Durham Co.; 9 samples from cottontails; larvae; 30-XI-1933, 2-XII-1933, 11-I-1934, 25-I-1934, 6-II-1934, 19-VII-1934, 25-XI-1934; adults: 21-IV-1934, 26-V-1934.	L, ♂, ♀
63	GEORGIA, Chatham Co., Savannah; rabbit, 1948; cottontail, 30-VIII-1942.	L, ♂, ♀
64	FLORIDA, Broward Co., Dania; 3 samples from *Sylvilagus palustris;* 29-V-1963.	L, ♂, ♀
65	VIRGINIA, Nottoway Co., Camp Pickett; 2 samples from cottontails; 2-IX-1960.	L
66	ALABAMA, Chambers Co., 3 m. W Cusseta; cottontails; 4-X-1963.	L
67	ALABAMA, Lee Co., Auburn University; larvae and adults: 3 samples from cottontails; 14-VIII-1963, 15-VIII-1963, 24-VIII-1963; larvae: 1 sample from quail; 27-IX-1963.	L, ♂, ♀
68	ALABAMA, Macon Co., Tuskegee Nat. For.; larvae and adults: 2 samples from cottontails; 16-IX-1963, 18-IX-1963; larvae: 2 samples from quail; 20-IX-1963.	L
69	OKLAHOMA, Lincoln Co.; *Sylvilagus floridanus;* 21-X-1964.	L
70	WYOMING, Natrona Co., Poison Spider Creek; jackrabbits; 25-VII-1935.	L
71	BRITISH COLUMBIA, Deep Creek, N end of Okanagan L., near Armstrong; rabbit; 10-V-1930.	L, ♂, ♀
72	ALASKA, Circle Hot Springs, Steese Highway; 4 samples from tree sparrows; 29-VIII-1964, 30-VIII-1964, 1-IX-1964.	L
73	ALASKA, Dot Lake, Alaskan Highway; *Lepus americanus;* 11-VII-1964.	L, ♂
74	ALASKA, Manley Hot Springs, Elliott Highway; fox sparrow; 10-VIII-1964; Lincoln sparrow; 12-VIII-1964; 2 white-crowned sparrows; 13-VIII-1964.	L

Table 1. List of Localities Employed in this Study (Concluded).

Locality code number	Locality, host and date	Life history stage
75	ALASKA, Fairbanks; Lincoln sparrow; 9-VIII-1964.	L
76	ALASKA, 30 m. S Nenana Highway; robin; 11-VII-1964.	L
77	ALBERTA, S of Manning. mile 181, MacKenzie Highway; *Lepus americanus;* 25-VIII-1950.	L
78	ALBERTA, Peace River; *Bonasa umbellus;* 27-VII-1947.	L
79	BRITISH COLUMBIA, Okanagan, N end Okanagan Lake; grouse; 15-X-1950.	L
80	BRITISH COLUMBIA, Oliver, 12 m. N British Columbia—Washington boundary, Hy. 97; rabbit; 30-V-1941.	L
81	ALBERTA, Medicine Hat; *Sylvilagus nuttallii;* 3-VII-1951.	L
82	SASKATCHEWAN, Estevan; rabbit; 18-VII-1942.	L
83	WASHINGTON, Pierce Co., Lake Tapps; *Lepus americanus;* 14-VIII-1939.	L
84	WASHINGTON, Lincoln Co., Odessa; pigmy rabbits; 9-VI-1949.	L
85	MONTANA, Lake Co.; cottontail; 16-X-1944.	L
86	MONTANA, Musselshell Co.; cottontail; 25-IV-1930.	L
87	MONTANA, Rosebud Co., Forsyth; cottontail; 11-VII-1930.	L
88	OREGON, Lake Co., 6 m. W Lakeview; jackrabbit; 20-V-1933.	L
89	WYOMING, Converse Co., Box Elder Canyon; cottontail; 5-VI-1934.	L, ♂, ♀
90	CALIFORNIA, Lassen Co., 10 m. SW Susanville; cottontail; 24-V-1933.	L
91	NEVADA, Elko Co., Elko; *Sylvilagus idahoensis;* 10-VIII-1961.	L
92	NEVADA, White Pine Co.; jackrabbit; 17-IV-1932.	L, ♂
93	NEVADA, Nye Co.; 2 samples from jackrabbits; 14-IV-1932, 15-IV-1932.	L
94	UTAH, Garfield Co.; 2 samples from rabbits; 23-VII-1936, 27-VII-1936.	L
95	COLORADO, Boulder Co.; cottontail; 27-IV-1940.	L
96	OKLAHOMA, Cimmarron Co., 10 m. E Kenton; *Sylvilagus* sp.; 24-X-1964.	L
97	CALIFORNIA, Riverside Co., Prado Dam Area; *Sylvilagus audubonii;* 1-X-1963.	L
98	ONTARIO, Wellington Co., Guelph; ruffed grouse; 1938?.	L
99	NEW YORK, Clinton Co., Valcour Island, Lake Champlain; *Lepus americanus;* 5-IV-1944.	L, ♂, ♀
100	NEW HAMPSHIRE, Grafton Co., Campton; ruffed grouse; 10-X-1952.	L
101	NEW HAMPSHIRE, Carroll Co., Passaconaway; *Lepus americanus;* 6-VIII-1959.	L
102	MAINE, Knox Co., Friendship; *Lepus americanus;* 5-VI-1958.	L
103	NEW YORK, Long Island, Montauk Point; cottontail; no date.	L
104	ILLINOIS, Iroquois Co.; cottontail; 25-X-1958.	L
105	WEST VIRGINIA, Hardy Co.; *Sylvilagus floridanus;* 28-X-1947.	L
106	VIRGINIA, Wise Co; cottontail; 20-II-1941.	L
107	TENNESSEE, Anderson Co., Oak Ridge; 2 samples from *Sylvilagus floridanus;* 11-X-1958, 24-II-1959.	L
108	GEORGIA, Clarke Co.; cottontail; 20-I-1952.	L
109	SOUTH CAROLINA, Beaufort Co., Pritchardville; tick drag; 3-XII-1943.	L
110	GEORGIA, Thomas Co.; rabbit; 1948. GEORGIA, Grady Co.; rabbit; 1948. FLORIDA, Leon Co.; catbird; prairie warbler; fall 1957.	L
111	ALABAMA, west Pickens Co.; 2 cottontails; 21-VIII-1963.	L
112	KENTUCKY, Nelson Co.; house sparrow; fall 1956. KENTUCKY, Green Co.; *Sylvilagus floridanus;* 2-IV-1959.	L
113	INDIANA, Ripley Co.; cottontail; 25-IV-1958.	L
114	INDIANA, Harrison Co.; *Thryothorus ludovicianus;* 8-III-1958.	L
115	INDIANA, Warwick Co.; *Sylvilagus aquaticus;* 29-X-1960.	L
116	ILLINOIS, White Co., Norris City; quail; 13-X-1938.	L
117	ILLINOIS, Jefferson Co., Mt. Vernon; 3 samples from cottontails; 17-IV-1956, 18-IV-1956.	L
118	SOUTH CAROLINA, Game Refuge; *Sylvilagus palustris;* 4-VI-1948.	L
119	ALBERTA?, McGill Lake; rabbit, 9-VI-1950.	L, ♂, ♀
120	UTAH, Juab Co., West Tintic Mountains; *Lepus californicus;* 21-IV-1961.	♂, ♀
121	NEW MEXICO, Santa Fe Co., Santa Fe; 3 samples from *Sylvilagus audubonii;* 11-III-1952, 18-IV-1952, 29-V-1952.	♂, ♀
122	ALABAMA, Lauderdale Co.; cottontail; 7-IX-1963.	L

Explanation: L, ♂, ♀ indicates that larvae, males and females, respectively, were measured from that locality.

TABLE 2. Means of Characters of Larvae from 64-Locality Study.

Locality code number	Characters and their code numbers							
	Width of scutum (1)	Length of scutum (2)	Length of tarsus I (3)	Length of coxa III (4)	Length of trochanter III (5)	Length of basifemur III (6)	Length of telofemur III (7)	Length of genu III (8)
1	368.8	240.3	210.6	143.3	110.9	32.1	111.1	126.6
2	371.5	243.6	211.4	140.8	113.8	33.9	114.0	131.3
3	375.9	244.1	210.6	146.8	115.5	32.5	116.5	132.0
4	377.7	246.1	212.7	144.2	110.8	31.0	112.2	127.0
5	366.9	235.3	213.1	144.1	113.0	33.4	114.3	130.3
6	369.3	245.3	201.3	138.3	109.1	32.6	109.1	122.0
7	365.1	231.7	195.7	141.8	102.7	31.2	112.2	132.6
8	347.1	222.3	190.9	136.3	99.3	31.4	111.0	127.9
9	364.9	227.9	194.5	137.7	102.7	32.4	110.3	129.2
10	332.7	213.7	196.5	125.9	92.9	28.8	98.1	122.1
11	360.4	221.9	199.7	140.3	98.3	30.3	108.7	132.5
12	356.3	223.5	198.4	136.1	100.6	31.3	107.4	127.1
13	358.4	223.3	199.0	140.9	101.7	32.0	109.8	129.7
14	366.0	227.1	200.4	139.9	99.8	30.1	109.4	130.3
15	348.9	222.8	197.0	130.7	100.6	30.7	109.1	128.5
16	320.3	204.4	175.9	113.3	87.1	29.5	93.1	111.9
17	311.7	203.1	184.0	112.7	86.5	27.9	91.2	110.8
18	334.1	228.8	189.0	128.2	96.3	30.6	104.5	123.7
19	324.9	213.5	189.6	127.0	94.2	27.1	98.5	124.6
20	349.2	218.3	198.3	138.3	97.2	32.7	109.0	125.1
21	363.6	227.1	202.5	141.3	104.2	31.9	113.5	132.4
22	359.3	225.7	196.3	136.9	99.1	30.4	110.0	132.9
23	359.6	229.7	200.8	140.7	102.9	30.8	110.6	129.1
24	351.1	216.1	196.7	136.9	102.3	32.3	109.5	125.7
25	367.5	236.5	209.9	149.1	112.4	31.5	113.9	129.3
26	358.7	222.9	191.1	135.6	99.1	31.1	107.8	126.2
27	352.4	220.0	192.7	135.0	99.9	29.7	107.7	127.7
28	331.1	226.5	199.5	127.7	102.7	32.9	110.0	131.2
29	372.3	238.7	212.0	148.7	112.9	31.5	113.8	130.3
30	363.2	234.5	209.9	138.1	110.2	29.9	111.8	130.1
31	362.2	235.7	207.9	141.6	111.1	32.1	113.1	128.5
32	363.6	238.7	208.5	140.6	110.5	30.7	111.7	128.2
33	353.1	228.0	200.2	134.5	105.9	29.7	107.4	123.5
34	374.3	241.3	221.3	147.9	115.9	32.9	116.6	132.9
35	362.4	233.3	206.5	138.6	111.1	31.2	113.7	128.9
36	363.1	231.2	202.9	140.0	110.7	31.3	113.4	127.3
37	323.1	211.3	197.5	125.5	100.0	30.8	103.9	121.8
38	343.8	223.6	208.8	139.9	105.0	34.1	109.8	126.5
39	330.9	222.7	203.2	132.0	106.3	31.9	111.2	128.1
40	337.9	223.9	205.8	137.9	107.5	32.5	109.5	126.7
41	335.5	224.5	204.1	137.7	106.1	32.7	108.9	124.4
42	337.3	219.7	190.8	133.7	101.7	30.9	105.8	121.6
43	352.0	235.6	207.0	135.1	105.1	32.9	112.3	131.6
44	335.9	226.8	197.7	131.6	98.7	29.2	106.5	127.9
45	319.5	209.6	189.4	126.5	95.5	29.5	98.4	114.3
46	342.1	219.7	208.4	135.2	108.5	34.0	109.6	124.7
47	364.8	243.5	203.3	140.3	115.5	34.7	116.8	127.5
48	367.9	240.0	210.3	141.8	114.6	34.5	115.6	130.2
49	371.1	248.7	215.8	146.1	115.7	35.1	118.1	133.9
50	321.9	217.6	195.3	125.2	101.1	31.9	105.1	121.5
51	319.5	213.7	197.5	126.8	101.7	31.2	106.1	123.2
52	328.0	220.8	199.6	126.1	101.2	31.9	106.4	124.2
53	343.5	228.9	210.3	133.5	108.4	32.9	111.3	127.5

TABLE 2. Means of Characters of Larvae from 64-Locality Study (Continued).

Locality code number	Width of scutum (1)	Length of scutum (2)	Length of tarsus I (3)	Length of coxa III (4)	Length of trochanter III (5)	Length of basifemur III (6)	Length of telofemur III (7)	Length of genu III (8)
54	337.5	226.5	203.2	136.9	106.1	33.1	109.9	127.5
55	336.7	228.4	200.6	131.1	105.8	33.7	108.9	124.7
56	368.7	235.7	216.3	148.1	113.7	32.9	117.0	134.3
57	362.1	242.7	202.3	142.8	113.5	37.2	114.3	128.5
58	347.3	238.3	209.9	138.7	111.1	35.3	115.3	128.5
59	331.7	226.0	202.9	138.3	106.0	31.7	110.3	127.4
60	331.3	222.4	208.7	137.5	108.5	32.3	111.1	132.3
61	332.0	226.3	205.9	132.6	108.2	33.3	111.7	127.1
62	336.0	227.5	201.6	131.1	107.3	32.3	109.6	125.8
63	322.8	219.5	196.1	128.1	104.1	32.5	106.5	123.5
64	330.1	226.7	197.7	129.6	106.2	32.6	108.9	125.9
Standard deviation	10.00	7.72	6.36	5.72	3.25	2.00	3.66	4.22

Locality code number	Length of tibia III (9)	Length of basitarsus III (10)	Length of telotarsus III (11)	Width of anal plate (12)	Width of basis capituli (13)	Length of palp (14)	Length of hypostome (15)	Length of chelicera (16)
1	101.5	63.1	120.6	63.4	139.2	111.3	72.5	242.0
2	102.8	65.0	121.5	65.7	140.0	112.0	74.6	243.9
3	103.5	64.2	121.9	63.7	140.7	110.3	75.6	245.1
4	102.9	64.7	125.0	65.3	144.3	109.3	70.4	256.3
5	104.6	66.1	122.1	64.9	142.9	110.1	74.3	241.2
6	96.7	60.9	116.3	61.0	136.7	108.5	70.3	236.0
7	102.0	61.4	115.8	61.9	132.2	103.1	70.4	230.0
8	99.1	58.7	112.4	60.2	129.4	102.1	67.6	229.3
9	99.3	60.5	114.7	62.6	130.3	104.2	69.1	232.1
10	95.5	58.5	115.2	54.4	131.8	89.6	62.8	229.8
11	103.2	63.0	119.5	60.0	129.7	100.5	66.9	223.2
12	96.7	56.3	113.0	57.3	128.9	97.3	66.1	225.2
13	99.5	60.7	117.8	59.4	131.3	104.0	68.8	231.5
14	99.3	61.5	119.1	60.2	131.5	102.7	67.7	230.7
15	98.1	59.9	116.4	60.3	126.0	100.5	67.0	224.5
16	87.8	53.4	102.9	53.5	124.9	87.7	65.4	220.7
17	87.5	52.6	107.3	50.3	124.8	86.5	63.6	223.5
18	95.5	57.0	113.9	57.7	126.3	101.7	69.9	236.0
19	95.7	54.2	107.3	54.7	123.0	95.1	70.5	226.7
20	97.1	59.0	117.2	55.5	136.4	106.8	75.3	236.9
21	102.1	61.7	117.6	60.9	133.5	106.1	68.9	234.1
22	101.4	62.0	115.9	59.9	128.1	102.9	68.8	229.5
23	99.9	60.5	116.3	60.7	133.0	103.7	66.1	241.5
24	94.5	58.5	116.6	58.5	134.9	103.1	64.8	230.4
25	100.2	62.5	122.3	61.1	141.1	110.9	75.3	244.5
26	97.5	58.1	111.9	58.5	128.2	102.4	69.2	229.9

TABLE 2. Means of Characters of Larvae from 64-Locality Study (Concluded).

Locality code number	Characters and their code numbers							
	Length of tibia III (9)	Length of basitarsus III (10)	Length of telotarsus III (11)	Width of anal plate (12)	Width of basis capituli (13)	Length of palp (14)	Length of hypostome (15)	Length of chelicera (16)
27	99.3	59.6	114.5	60.5	131.3	100.4	68.5	228.4
28	101.9	62.1	115.6	58.9	120.5	103.6	73.9	229.1
29	104.0	65.1	122.3	63.4	142.3	111.7	72.2	244.7
30	102.9	63.9	120.6	63.8	138.3	108.6	73.4	233.5
31	100.6	63.1	117.5	64.5	139.1	109.0	72.2	238.3
32	101.7	61.9	119.7	62.0	139.5	109.9	71.9	242.1
33	96.4	61.7	113.9	61.0	133.3	105.7	72.5	232.4
34	104.1	64.9	127.5	61.7	150.5	116.6	71.1	247.1
35	99.7	62.3	116.7	63.9	139.0	108.9	71.8	236.1
36	100.8	63.7	119.9	63.3	138.3	110.3	69.9	232.3
37	96.5	58.3	111.0	56.2	126.9	107.2	69.6	231.9
38	98.3	60.7	120.1	58.9	132.7	117.1	73.2	248.0
39	100.1	59.7	116.4	58.3	130.3	112.2	73.1	235.7
40	99.4	59.4	117.1	57.4	133.3	112.7	73.0	242.7
41	98.1	60.5	117.8	58.9	131.3	112.9	69.1	234.3
42	95.0	55.9	110.1	54.4	127.9	107.5	69.3	230.3
43	98.2	62.1	121.9	55.0	126.6	103.2	72.1	235.9
44	98.4	59.2	117.1	55.5	121.3	98.1	67.3	226.3
45	89.1	54.3	108.3	53.7	125.1	104.6	67.5	228.1
46	100.1	58.6	115.6	53.3	134.2	113.0	71.4	233.6
47	101.1	62.0	113.5	66.1	140.2	112.3	77.5	242.7
48	101.7	61.9	117.3	65.3	140.7	110.5	76.6	243.7
49	106.1	65.1	125.8	65.4	145.9	117.1	73.2	247.9
50	94.3	57.1	109.5	57.4	128.7	106.6	70.3	231.3
51	95.9	58.5	112.3	54.7	128.5	106.7	69.4	232.5
52	98.4	60.0	114.7	59.1	130.2	108.3	70.7	230.1
53	102.4	62.7	120.0	57.4	133.7	113.5	71.6	237.2
54	99.5	59.7	115.1	58.2	133.2	113.1	75.4	244.3
55	98.0	57.9	113.4	55.9	131.0	114.1	74.3	243.3
56	106.3	65.6	126.0	64.4	142.7	114.9	75.4	250.7
57	99.8	59.1	113.3	68.5	137.5	111.8	77.7	243.6
58	101.9	62.7	118.4	64.1	138.7	117.2	78.7	252.7
59	98.9	61.0	118.6	59.5	132.1	114.7	72.9	242.3
60	103.9	63.0	119.1	57.5	131.9	111.4	73.4	236.8
61	100.3	62.2	115.9	60.6	129.9	112.9	72.7	231.2
62	99.7	60.9	116.1	58.9	130.3	111.5	72.9	241.5
63	96.3	58.0	111.3	56.5	126.8	109.3	72.6	237.1
64	99.3	60.7	114.2	57.7	128.1	111.3	73.1	239.1
Standard deviation	3.84	3.14	4.75	3.01	4.10	3.58	2.73	7.72

EXPLANATION: All means are given in microns. Sample size is uniformly 15. The standard deviation at the foot of each column is the square root of the error mean square (the average standard deviation within localities). It is *not* the standard error of the mean. In order to obtain an average standard error for these means the standard deviation can be divided by $\sqrt{15}$, the square root of the sample size.

TABLE 3. Means of Characters of Larvae from 120-Locality Study.

		Characters and their code numbers					
Locality code number	Sample size	Width of scutum (1)	Length of genu III (8)	Length of tibia III (9)	Width of basis capituli (13)	Length of palp (14)	Length of hypostome (15)
1	15	368.8	126.6	101.5	139.2	111.3	72.5
2	15	371.5	131.3	102.8	140.0	112.0	74.6
3	15	382.1	131.7	103.3	142.1	111.9	74.9
4	15	377.7	127.0	102.9	144.3	109.3	70.4
5	15	366.9	130.3	104.6	142.9	110.1	74.3
6	15	369.3	122.0	96.7	136.7	108.5	70.3
7	15	365.1	132.6	102.0	132.2	103.1	70.4
8	15	351.9	128.7	99.3	128.9	100.4	68.3
9	15	360.7	129.5	99.5	131.6	104.2	68.7
10	15	332.7	122.1	95.5	131.8	89.6	62.8
11	15	357.2	132.5	103.6	130.7	101.9	67.6
12	15	359.1	129.7	98.8	129.4	100.4	68.7
13	15	360.7	133.3	103.5	132.5	103.0	69.3
14	15	366.0	130.3	99.3	131.5	102.7	67.7
15	15	348.9	128.5	98.1	126.0	100.5	67.0
16	15	318.8	111.9	87.4	124.1	87.5	65.9
17	15	311.7	110.8	87.5	124.8	86.5	63.6
18	15	334.1	123.7	95.5	126.3	101.7	69.9
19	15	324.9	124.6	95.7	123.0	95.1	70.5
20	15	349.2	125.1	97.1	136.4	106.8	75.3
21	15	363.6	132.4	102.1	133.5	106.1	68.9
22	15	359.3	132.9	101.4	128.1	102.9	68.8
23	15	359.6	129.1	99.9	133.0	103.7	66.1
24	15	351.1	125.7	94.5	134.9	103.1	64.8
25	15	367.5	129.3	100.2	141.1	110.9	75.3
26	15	358.7	126.2	97.5	128.2	102.4	69.2
27	15	352.5	129.7	100.6	130.6	101.9	69.3
28	15	331.1	131.2	101.9	120.5	103.6	73.9
29	15	372.3	130.3	104.0	142.3	111.7	72.2
30	15	363.2	130.1	102.9	138.3	108.6	73.4
31	15	367.3	128.7	103.1	142.1	110.3	73.4
32	15	360.5	129.0	101.4	140.9	109.7	71.6
33	15	357.7	125.7	99.1	138.5	108.0	71.8
34	15	371.7	131.7	103.5	145.8	114.1	71.4
35	15	364.3	128.4	101.0	139.5	109.1	72.4
36	15	365.9	129.4	102.7	141.1	111.3	71.2
37	15	321.9	122.2	96.2	126.7	107.9	70.1
38	15	338.0	124.0	96.1	131.9	112.4	71.7
39	15	330.9	128.1	100.1	130.3	112.2	73.1
40	15	339.3	127.7	98.7	132.1	112.5	73.2
41	15	335.5	124.4	98.1	131.3	112.9	69.1
42	15	337.3	121.6	95.0	127.9	107.5	69.3
43	15	352.0	131.6	98.2	126.6	103.2	72.1
44	15	333.9	124.8	96.1	123.5	101.4	68.9
45	15	319.5	114.3	89.1	125.1	104.6	67.5
46	15	342.1	124.7	100.1	134.2	113.0	71.4
47	15	364.8	127.5	101.1	140.2	112.3	77.5
48	15	367.9	130.2	101.7	140.7	110.5	76.6
49	15	372.3	132.7	103.3	148.3	116.9	73.2
50	15	325.0	124.1	98.9	130.3	109.0	69.2
51	15	319.5	123.2	95.9	128.5	106.7	69.4
52	15	328.5	123.9	98.7	130.5	108.0	71.3
53	15	343.5	127.5	102.4	133.7	113.5	71.6

TABLE 3. Means of Characters of Larvae from 120-Locality Study (Continued).

		Characters and their code numbers					
Locality code number	Sample size	Width of scutum (1)	Length of genu III (8)	Length of tibia III (9)	Width of basis capituli (13)	Length of palp (14)	Length of hypostome (15)
54	15	330.5	127.3	99.5	132.2	111.9	72.0
55	15	326.9	124.2	97.9	129.9	111.6	71.0
56	15	368.7	134.3	106.3	142.7	114.9	75.4
57	15	361.1	126.9	98.9	138.8	110.9	75.7
58	15	346.8	128.2	101.4	138.7	116.7	78.7
59	15	331.7	127.4	98.9	132.1	114.7	72.9
60	15	332.5	125.3	97.3	129.5	111.0	71.3
61	15	332.0	127.1	100.3	129.9	112.9	72.7
62	15	334.7	126.2	99.2	132.2	112.8	72.5
63	15	322.8	123.5	96.3	126.8	109.3	72.6
64	15	330.1	125.1	98.7	128.8	109.4	71.2
65	15	323.2	123.4	97.7	127.5	111.3	69.9
66	15	331.3	122.3	95.9	126.2	110.3	71.0
67	15	340.5	128.6	100.5	130.2	111.3	71.4
68	15	337.0	128.3	101.5	130.1	111.5	70.7
69	15	346.3	123.7	98.4	130.5	111.9	71.3
70	15	359.9	127.3	99.7	128.5	99.9	69.1
71	15	379.7	131.2	100.9	146.4	114.0	75.7
72	15	368.7	128.2	101.3	141.9	114.0	73.1
73	15	380.9	129.9	103.9	147.1	115.5	71.6
74	15	382.6	129.6	102.5	146.7	115.5	76.6
75	5	373.4	126.8	100.8	141.6	112.4	72.6
76	3	374.0	123.7	98.0	138.7	113.0	73.3
77	3	374.0	130.3	105.3	145.3	112.7	73.3
78	2	369.0	127.5	107.0	145.0	110.0	67.5
79	9	377.8	133.2	103.0	146.4	117.0	74.7
80	11	349.7	130.3	101.1	130.0	101.8	68.6
81	3	372.7	130.0	100.0	137.7	109.0	69.3
82	9	372.0	126.6	99.9	140.9	112.2	74.0
83	7	374.0	137.1	105.7	143.7	116.7	78.7
84	7	352.7	131.6	101.0	129.9	101.1	69.1
85	10	351.8	130.6	101.6	133.9	105.5	71.3
86	4	349.8	130.0	99.5	130.8	103.0	69.8
87	8	347.5	122.4	93.4	131.8	99.8	64.4
88	5	363.6	133.2	102.4	132.0	101.2	69.0
89	5	361.2	130.2	101.4	135.2	100.4	65.0
90	7	376.3	133.1	105.4	135.1	106.3	69.7
91	11	357.7	129.7	98.3	132.3	100.9	71.0
92	3	364.7	136.0	101.7	129.7	102.7	67.3
93	4	356.0	131.0	102.2	129.3	106.3	70.0
94	14	349.1	128.2	99.4	128.5	100.4	67.1
95	9	360.4	133.7	103.4	131.8	105.2	72.2
96	4	349.5	125.3	97.5	129.0	103.5	69.5
97	5	334.6	126.6	95.4	124.6	96.6	69.4
98	11	379.9	136.7	106.9	151.0	118.3	75.9
99	3	368.3	127.7	100.7	140.0	108.7	76.3
100	3	363.7	128.0	97.7	144.3	113.7	70.0
101	5	377.8	132.0	101.4	146.0	116.2	76.6
102	2	331.5	127.0	93.0	131.0	103.0	68.5
103	10	333.4	125.4	97.1	131.1	112.2	75.9
104	10	335.1	125.3	97.9	131.4	111.7	69.0
105	2	343.5	121.5	97.5	127.0	110.5	72.5
106	8	341.6	127.5	98.8	135.5	115.4	74.8

TABLE 3. Means of Characters of Larvae from 120-Locality Study (Concluded).

Locality code number	Sample size	Width of scutum (1)	Length of genu III (8)	Length of tiba III (9)	Width of basis capituli (13)	Length of palp (14)	Length of hypostome (15)
		Characters and their code numbers					
107	3	341.0	122.7	93.7	129.7	112.0	69.7
108	2	347.5	119.5	92.0	132.0	111.5	69.5
109	7	333.1	125.4	99.4	129.0	111.3	72.0
110	12	328.8	123.3	96.1	127.5	108.2	71.5
111	3	341.3	129.7	101.7	133.3	113.3	71.7
112	3	338.7	126.0	100.7	132.0	111.0	75.0
113	13	340.4	128.9	101.1	136.5	114.0	73.1
114	6	327.3	129.8	100.8	126.0	112.0	72.8
115	9	342.8	128.4	101.2	135.2	116.3	74.9
116	6	328.2	123.7	96.0	126.7	109.5	68.8
117	6	355.2	127.8	97.3	132.3	113.7	71.3
118	12	344.8	129.8	100.7	132.1	117.1	75.1
119	15	376.5	130.8	102.1	143.5	113.4	77.7
122	1	334.0	126.0	97.0	127.0	111.0	71.0
Standard deviation		10.39	4.26	3.68	4.42	3.67	2.70

EXPLANATION: All means are given in microns. The standard deviation at the foot of each column is the square root of the error mean square (the average standard deviation within localities). It is *not* the standard error of the mean. In order to obtain an approximate standard error for these means the standard deviation can be divided by the square root of the average number of ticks per locality ($\sqrt{11.734}$), calculated as $n_0 = \frac{1}{a-1}\left[\sum^{a} n_i - \left(\sum^{a} n_i^2 / \sum^{a} n_i\right)\right]$ where a is the number of localities and n_i is the number of ticks from the ith locality.

TABLE 4. Means of Characters of Males from 38-Locality Study.

		Characters and their code numbers					
Locality code number	Sample size	Width of scutum (1)	Length of genu III (8)	Length of tibia III (9)	Width of basis capituli (13)	Length of palp (14)	Length of hypostome (15)
3	15	1011	278	221	306	290	171.0
4	15	1041	277	231	310	292	167.2
10	7	967	271	218	259	231	153.4
11	15	959	266	219	261	240	138.4
13	15	959	267	216	259	239	140.9
17	15	742	199	161	216	206	121.2
18	4	938	264	214	252	238	140.0
24	8	923	252	202	252	236	135.2
33	15	934	262	205	293	280	164.5
37	15	872	254	210	238	239	131.5
38	15	886	245	205	235	242	127.6
39	15	905	255	213	243	249	137.5
40	15	836	244	197	236	249	139.6
42	7	1018	305	253	275	266	172.8
43a	10	1164	340	283	315	295	192.8
43b	10	1129	330	275	298	280	181.4
47	15	1023	272	227	302	286	165.7
48	15	965	274	222	299	289	167.6
49	7	1033	269	220	297	285	166.8
50	15	913	262	213	247	249	135.1
52	15	922	263	214	240	240	130.7
54	15	894	253	208	243	253	142.0
55	15	869	252	209	244	257	140.9
57	15	973	270	225	300	281	166.1
58	15	926	262	219	271	278	157.9
60	6	849	250	202	239	253	143.3
62	15	913	254	207	252	258	137.9
63	15	857	250	205	238	248	140.8
64	15	806	237	195	227	240	129.3
67	15	819	241	190	235	245	138.8
71	15	1068	282	234	315	299	167.3
73	7	1043	275	229	307	287	162.9
89	5	893	251	207	250	231	137.6
92	10	957	256	208	250	226	134.0
99	15	945	269	222	297	289	172.1
119	15	1020	275	234	305	293	167.6
120	15	1017	276	227	270	250	145.1
121	7	967	260	215	263	245	141.4
Standard deviation		45.9	13.0	10.9	9.5	10.0	7.17

EXPLANATION: All means are given in microns. The standard deviation at the foot of each column is the square root of the error mean square (the average standard deviation within localities). It is *not* the standard error of the mean. In order to obtain an approximate standard error for these means the standard deviation can be divided by the square root of the average number of ticks per locality ($\sqrt{12.549}$), calculated as $n_0 = \frac{1}{a-1}\left[\sum^{a} n_i - \left(\sum^{a} n_i^2 \Big/ \sum^{a} n_i\right)\right]$ where a is the number of localities and n_i is the number of ticks from the ith locality.

TABLE 5. Means of Characters of Females from 36-Locality Study.

		Characters and their code numbers					
Locality code number	Sample size	Width of scutum (1)	Length of genu III (8)	Length of tibia III (9)	Width of basis capituli (13)	Length of palp (14)	Length of hypostome (15)
3	15	927	362	262	474	454	278
4	5	953	368	283	480	460	279
10	5	808	338	249	392	356	230
11	9	802	334	264	398	378	216
13	2	808	355	262	408	384	212
17	15	688	265	215	346	329	198
18	5	792	340	254	410	394	232
24	10	732	302	235	370	349	193
33	15	871	338	275	452	432	260
37	4	733	313	254	383	399	221
38	1	740	306	248	404	400	254
39	3	790	324	263	410	422	261
40	15	765	311	248	398	410	249
42	8	738	292	245	370	393	245
43a	10	968	410	333	466	433	276
43b	10	926	394	315	477	407	261
47	15	920	359	294	473	449	267
48	15	888	352	278	463	440	265
49	5	915	357	277	467	439	263
50	15	810	327	264	406	412	226
52	9	808	336	275	409	408	229
54	15	807	328	261	411	425	257
55	15	784	320	262	418	423	253
57	15	900	357	292	478	445	267
58	12	869	342	269	441	442	273
60	6	788	323	263	414	429	266
62	15	796	330	267	432	430	250
63	15	751	313	252	396	400	247
64	15	737	305	248	395	403	245
67	8	783	317	254	400	417	267
71	15	961	369	294	500	461	270
89	4	784	328	264	392	374	221
99	10	875	349	285	455	435	270
119	15	928	358	293	473	444	266
120	7	842	348	280	417	386	225
121	4	822	332	256	409	387	222
		38.3	14.1	12.9	14.7	16.1	10.6

EXPLANATION: All means are given in microns. The standard deviation at the foot of each column is the square root of the error mean square (the average standard deviation within localities). It is *not* the standard error of the mean. In order to obtain an approximate standard error for these means the standard deviation can be divided by the square root of the average number of ticks per locality ($\sqrt{9.991}$), calculated as $n_0 = \frac{1}{a-1}\left[\sum^{a} n_i - \left(\sum^{a} n_i^2 \Big/ \sum^{a} n_i\right)\right]$ where a is the number of localities and n_i is the number of ticks from the ith locality.

TABLE 6. Coordinates of Tick Populations (Localities) on Three Principal Axes.

Locality code number	Factor			Locality code number	Factor		
	I	II	III		I	II	III
1	1.46	–0.08	0.86	38	0.44	1.38	–0.04
2	2.42	–0.13	0.27	39	0.09	1.15	–0.88
3	2.73	–0.30	0.26	40	0.26	1.09	–0.20
4	1.88	–0.94	1.47	41	–0.69	0.74	0.01
5	2.51	–0.21	0.49	42	–1.83	0.59	0.28
6	–0.12	–0.20	1.61	43	–0.22	–0.51	–1.16
7	0.85	–1.40	–0.71	44	–2.01	–0.97	–1.48
8	–0.88	–1.00	–0.47	45	–4.17	1.30	1.07
9	–0.01	–1.19	–0.24	46	0.16	0.85	0.20
10	–3.24	–1.89	0.65	47	2.03	0.90	0.72
11	0.21	–2.07	–1.08	48	2.26	0.30	0.47
12	–1.51	–1.75	–0.03	49	3.61	–0.25	0.34
13	–0.06	–1.11	–0.33	50	–2.16	1.16	0.12
14	–0.03	–1.65	–0.20	51	–1.96	0.91	–0.26
15	–1.29	–1.26	–0.78	52	–1.02	0.85	–0.28
16	–5.73	–0.25	1.50	53	0.76	0.61	–0.38
17	–6.31	–0.33	1.54	54	0.59	1.47	–0.38
18	–2.00	0.17	–0.27	55	–0.09	1.66	–0.13
19	–2.64	–0.06	–0.94	56	3.51	–0.01	–0.16
20	0.16	0.84	0.69	57	1.70	0.94	0.35
21	0.91	–1.36	–0.54	58	2.14	1.91	0.02
22	0.23	–1.50	–1.21	59	0.14	1.39	–0.49
23	–0.22	–1.60	–0.02	60	1.06	0.71	–1.54
24	–1.44	–1.21	0.91	61	0.02	1.18	–0.80
25	1.89	0.23	0.77	62	–0.12	1.11	–0.46
26	–0.89	–0.85	–0.07	63	–1.45	1.54	–0.52
27	–0.63	–1.13	–0.18	64	–0.43	1.29	–0.80
28	–0.46	0.36	–2.49	65	–1.62	1.17	–0.40
29	2.40	–0.55	0.61	66	–1.83	1.21	–0.11
30	1.70	–0.27	0.11	67	–0.02	0.47	–0.79
31	1.21	–0.23	0.57	68	–0.05	0.44	–0.90
32	1.40	–0.26	0.59	69	–0.61	0.79	0.17
33	–0.53	0.34	0.74	70	–0.88	–1.35	–0.32
34	3.39	–0.63	1.22	71	2.56	0.41	0.90
35	1.07	–0.32	0.60	72	1.75	0.18	0.87
36	0.92	–0.46	0.68	73	2.79	–0.56	1.18
37	–1.98	0.97	–0.25	74	3.13	0.37	1.23

Table 7. Multiple Regression Equations of Characters of Larval Ticks on Environmental Variables.

	Dependent Variables					
Independent Variables	Width of scutum (1)	Length of genu III (8)	Length of tibia III (9)	Width of basis capituli (13)	Length of palp (14)	Length of hypostome (15)
Mean total precipitation M-2	–3.58			–1.16		–0.68
M-1	4.63					
Month of collection		1.58	0.85		2.11	1.25
Annual	–0.39	–0.17				–0.09
Mean temperature M-2						
M-1						
Month of collection						
Annual		0.28				–0.11
Mean maximum temperature M-2						
M-1						
Month of collection					–0.10	
Annual				–0.54		
Mean minimum temperature M-2						
M-1						
Month of collection						
Annual					0.49	
Altitude				–0.001	–0.002	–0.001
Latitude						
Longitude				–0.14		
Isophane	1.39	0.62	0.32		1.14	–0.10
Y-intercept	292.90	85.49	82.53	185.37	41.33	88.56
Coefficient of multiple correlation	0.82	0.74	0.73	0.84	0.84	0.68
Percentage of determination	67.83	54.85	53.61	70.50	70.62	45.67

Explanation: All weather data are based on long term averages obtained from U. S. Weather Bureau publications. Elevation is in feet above sea level, latitude in °N, longitude in °W, and isophane in °N. M-2 and M-1 refer to two months and one month prior to month of collection (M) of tick specimens. The values under each dependent variable are the significant ($P \leqq 0.05$) partial regression coefficients of that character on the corresponding environmental variable.

TABLE 8. Correlations of Larval Characters and of Environmental Variables.

		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Total precipitation M-2	(1)	X									
M-1	(2)	77	X								
M	(3)	69	83	X							
Annual	(4)	59	63	72	X						
Mean temperature M-2	(5)	*30*	*26*	*14*	*32*	X					
M-1	(6)	*23*	*19*	*11*	*17*	86	X				
M	(7)	*08*	*09*	*11*	*08*	40	77	X			
Annual	(8)	*10*	*10*	*20*	51	*31*	*26*	38	X		
Mean maximum temperature M-2	(9)	*18*	*15*	*–01*	*20*	95	81	36	*26*	X	
M-1	(10)	*06*	*00*	*–11*	*02*	76	91	71	*20*	85	X
M	(11)	*11*	*12*	*09*	*11*	*18*	42	51	*11*	*21*	46
Annual	(12)	*01*	*00*	*09*	41	*29*	*25*	39	99	*26*	*23*
Mean minimum temperature M-2	(13)	40	38	*22*	42	91	75	34	36	89	69
M-1	(14)	38	37	*24*	*32*	82	91	70	*31*	82	88
M	(15)	*24*	*29*	*26*	*24*	40	72	93	42	39	68
Annual	(16)	*19*	*20*	*29*	59	34	*26*	35	99	*26*	*17*
Altitude	(17)	–38	–41	–45	–57	*–22*	*–07*	*–01*	*–32*	*–12*	*07*
Latitude	(18)	*–15*	*–13*	*–17*	–46	*–28*	*–22*	*–31*	–94	*–23*	*–16*
Longitude	(19)	–69	–66	–60	–72	*–22*	*–08*	*–02*	–43	*–11*	*06*
Isophane	(20)	*–05*	*–07*	*–17*	–47	*–30*	*–22*	*–31*	–92	*–25*	*–15*
Width of scutum	(1L)	*–26*	*–13*	*–24*	–57	*–25*	*–18*	*–24*	–75	*–21*	*–13*
Length of genu III	(8L)	*–02*	*10*	*07*	–34	*–22*	*–14*	*–14*	–47	*–20*	*–12*
Length of tibia III	(9L)	*16*	*28*	*23*	–17	*–14*	*–11*	*–20*	–55	*–15*	*–14*
Width of basis capituli	(13L)	*06*	*20*	*09*	–15	*–15*	*–16*	*–29*	–73	*–17*	*–20*
Length of palp	(14L)	50	64	63	39	*05*	*–05*	*–18*	*–20*	*–06*	*–20*
Length of hypostome	(15L)	*30*	47	48	*20*	*–09*	*–13*	*–13*	*–19*	*–13*	*–21*

TABLE 8. Correlations of Larvel Characters and Environmental Variables (Concluded).

	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(1L)	(8L)	(9L)	(13L)	(14L)	(15L)
(1)																
(2)																
(3)																
(4)																
(5)																
(6)																
(7)																
(8)																
(9)																
(10)																
(11)	X															
(12)	*12*	X														
(13)	*18*	*31*	X													
(14)	43	*27*	86	X												
(15)	51	40	47	80	X											
(16)	*10*	95	41	35	44	X										
(17)	*19*	*–21*	*–32*	*–22*	*–17*	–43	X									
(18)	*–11*	–94	*–31*	*–26*	–35	–91	*12*	X								
(19)	*–03*	–36	*–32*	*–22*	*–17*	–50	*30*	53	X							
(20)	*00*	–89	–35	*–28*	–38	–93	54	*25*	*25*	X						
(1L)	*–06*	–72	*–31*	*–27*	*–32*	–77	39	38	38	75	X					
(8L)	*–02*	–44	*–26*	*–21*	*–20*	–49	42	*04*	*04*	61	73	X				
(9L)	*–07*	–56	*–16*	*–14*	*–21*	–53	*26*	*–05*	*–05*	65	71	91	X			
(13L)	*–15*	–77	*–14*	*–15*	*–27*	–67	*–07*	*09*	*09*	64	76	46	62	X		
(14L)	*–17*	*–29*	*13*	*06*	*–05*	*–12*	*–27*	–47	–47	*27*	*26*	40	58	57	X	
(15L)	*–15*	*–25*	*–03*	*–05*	*–03*	*–14*	*–26*	–32	–32	*19*	*29*	40	52	49	74	X

EXPLANATION: Correlation coefficients not significant at P ≦ 0.01 are in italics ($r \leqq 0.32$). Decimal poin have been omitted. See Table 7 for explanation of environmental variables.

TABLE 9. Primary Patterns and Correlation among Factors Based on Correlations of Environmental Variables and Larval Characters.

		I	II	III	IV	V	VI	h^2
otal precipitation M-2	(1)	86	11	13	–01	43	–26	74
M-1	(2)	77	03	05	10	74	–04	83
Month of collection	(3)	70	–16	–06	15	76	–18	80
Annual	(4)	65	05	–22	–08	37	–74	78
ean temperature M-2	(5)	06	98	–07	–06	06	–03	95
M-1	(6)	01	65	04	50	–01	14	93
Month of collection	(7)	–08	–04	–11	97	–04	–01	94
Annual	(8)	–05	02	–96	08	00	–1.01	1.00
ean maximum temperature M-2	(9)	04	1.03	–05	–07	–06	03	97
M-1	(10)	–11	68	05	47	–21	19	94
Month of collection	(11)	25	–06	08	55	–23	–03	42
Annual	(12)	–13	02	–1.00	08	–09	–97	99
ean minimum temperature M-2	(13)	17	89	–06	–05	14	–15	90
M-1	(14)	18	62	09	48	08	–01	97
Month of collection	(15)	08	–04	–07	95	09	–13	95
Annual	(16)	03	03	–87	07	09	–1.03	98
ltitude	(17)	–05	–05	–11	–01	–45	50	84
atitude	(18)	–13	–03	99	04	09	1.01	95
ongitude	(19)	–72	–04	44	18	–53	48	77
ophane	(20)	22	–02	71	–09	03	1.04	97
Width of scutum	(1L)	–34	04	37	04	46	1.34	86
Length of genu III	(8L)	–08	–03	–15	04	77	1.22	89
Length of tibia III	(9L)	08	04	04	–02	90	1.23	90
Width of basis capituli	(13L)	–02	06	64	–02	66	1.05	79
Length of palp	(14L)	39	06	08	–09	1.09	57	82
Length of hypostome	(15L)	13	–09	00	05	1.03	61	62

Factors	I	II	III	IV	V	VI
I	X	06	–45	13	–33	47
II	06	X	01	49	09	–24
III	–45	01	X	–14	54	–52
IV	13	49	–14	X	–03	–14
V	–33	09	54	–03	X	–65
VI	47	–24	–52	–14	–65	X

XPLANATION: The upper matrix gives the magnitude of the primary pattern coefficients (decimal points mitted except where the value is equal to or greater than one). The communality (h^2) is the percentage f variation due to common factors for each character. The lower matrix gives the correlations of the six ctors. M-2 and M-1 refer to two months and one month prior to month of collection (M) of specimens.

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VARIATION AND COVARIATION IN CHARACTERS OF THE RABBIT TICK, *HAEMAPHYSALIS LEPORISPALUSTRIS*

By

Paul A. Thomas

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THE UNIVERSITY OF KANSAS
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Variation and Covariation in Characters of the Rabbit Tick, *Haemaphysalis Leporispalustris*[1,2]

PAUL A. THOMAS[3,4]
Department of Entomology
The University of Kansas

ABSTRACT

The variation and covariation of morphological characters of the rabbit tick, *H. leporispalustris,* were investigated for 122 localities distributed from Fairbanks, Alaska, south to San Diego County, California, southeast to Brownsville, Texas, and Broward County, Florida, and north to Fredericton, New Brunswick. Separate studies of variation and covariation in larvae, males and females were considered for 64, 38, and 36 localities respectively. In addition the concordance of geographic variation of the three stages was examined for 33 localities. The study involved 39,386 measurements of 3,076 larvae, 478 males and 362 females.

Three studies of interlocality variation of larval characters composed of samples of 33, 64 and 120 localities showed significant added variance among localities for all characters and comparable estimates of variance components for the same characters in all three studies. Six characters of males and females showed greater variance within and among localities than homologous characters of larvae. Several possible explanations are discussed for this phenomenon.

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Statistical computations were carried out at The University of Kansas Computation Center.

[3] Present address: Air Force Armament Laboratory, Eglin AFB, Florida.

[4] I wish to express my appreciation to the following for their assistance in connection with this study: To Dr. Robert R. Sokal for introducing me to statistics and its application in studies of geographic variation and for his advice and encouragement throughout this study; to Dr. Joseph H. Camin for proposing the use of the rabbit tick and for his interest and valuable suggestions; to Dr. Richard Johnston for reading and commenting on the manuscript; to Dr. F. James Rohlf for providing helpful advice on computational methods; to Dr. Glen M. Kohls and many other workers listed in Thomas (1965), who generously loaned tick specimens; to Dr. Harold Willis for photographic assistance; and, finally, to my wife, Judith, for her patience throughout this study and for her assistance with the preparation of the figures.

The percentages of added variance among host individuals of six larval characters from 30 different localities varied from 0 to 82.8%. No one character was more variable than any other and larvae from about half of the localities showed significant variation among host individuals within localities. These results were interpreted in the light of behavioral patterns of ticks and hosts. Variation of larval characters among host species and among host individuals of the same species was investigated for 12 localities. Larvae from resident host species showed no added variation among host species. Localities with samples including migratory birds as hosts yielded added variation of larval characters among host species above and beyond variation among host individuals suggesting that migratory birds play a role in dispersal of tick populations. Larvae from host species having a wide distribution have significant variation among localities in excess of variation among ticks from different host individuals within a locality.

Correlations of tick characters were computed as product-moment coefficients within localities and as product-moment and component coefficients among localities. These three types of correlation matrices were obtained for larval characters in 64 and 33 localities, for male characters in 38 localities and for female characters in 36 localities. The intralocality correlations were lower in magnitude than the two interlocality matrices but showed related patterns of correlation. The interlocality component and product-moment matrices were essentially identical. Principal axis factor analysis with rotation to simple structure explained the covariation in terms of fewer variables (factors). Adjacent or functionally related characters were influenced by the same factor indicating that the patterns of correlations appeared to be morphologically localized. Intralocality and interlocality factors showed similar relationships to characters within a life history stage. Patterns of covariation in larvae were more similar to those of females than of males, emphasizing the greater morphological similarity between the first two life history stages. Factor analysis of the interlocality component matrix of 16 characters from 64 localities resulted in three independent trends of variation which were represented by six characters measured in subsequent studies. The same six characters were studied in males and females to allow comparisons of variation among the different life history stages. Concordance of patterns of variation of larvae, males, and females was investigated by correlating mean values of six characters of each morphotype for 33 localities. Factor analysis of this matrix yielded four factors, three corresponding to previously discovered larval factors and a new "adult" factor influencing only adult characters.

INTRODUCTION

This work is a statistical analysis of the amounts and sources of variation and covariation of morphological characters of the rabbit tick, *Haemaphysalis leporispalustris* (Packard). It is based upon 39,386 measurements of 3,076 larvae, 478 males and 362 females from preserved tick material obtained primarily from laboratories and museums throughout North America. Ticks from 122 localities distributed from Fairbanks, Alaska, south to San Diego

County, California, southeast to Brownsville, Texas, and Broward County, Florida, and north to Fredericton, New Brunswick, were studied.

Since the development of the New Systematics, variation of many species has been studied extensively at the population level. Analysis of variation and covariation of characters within and among local populations over the range of a species provides valuable clues to the operation of evolutionary mechanisms. This study of variation of *H. leporispalustris* was designed to achieve four main objectives: (1) demonstration of the use of statistical methods in variation studies; (2) description of variation of males, females and larvae with and among populations; (3) study of concordance of variation patterns of males, females and larvae and among various characters of these life history stages; (4) explanation of observed variation and covariation in terms of possible causal factors such as physiography, climate, and the life history, ecology, and behavior of ticks and hosts.

A description of geographic variation patterns, categorization of possible infraspecific units, and an analysis of the relationship of some putative causal variables to the observed variation of ticks is presented in a companion paper (Thomas, 1967).

Haemaphysalis leporispalustris is distributed throughout the New World from Alaska to Argentina in areas ecologically suitable for its principal hosts, cottontails and hares (genera *Sylvilagus* and *Lepus*). Owing to scarcity of material from Central and South America, the area of this study was restricted to North America north of Mexico.

The life cycle of *H. leporispalustris* is typical of an ixodid tick. The egg hatches into a hexapod larva which lies in wait for a suitable host, usually a rabbit or a bird. The larva attaches to the host and feeds upon blood until engorged. It then drops from the host and makes its way into ground litter where it molts to become an octopod nymph. After the cuticle of the nymph has hardened, it will attach upon any suitable passing host, feed again until engorgement and then drop off. The nymph molts into either a male or a female tick which awaits the passage of another host. The adults are more host-specific than larvae or nymphs and will usually feed only on cottontails or hares. Copulation occurs while the female is attached to the host. When the female finishes feeding, it drops from the host and after several days lays a cluster of eggs, thus completing the cycle. The female does not feed again, and dies shortly after oviposition.

MATERIALS AND METHODS

a. Materials

The main emphasis of this study is on larvae because they were the most abundant life history stage and specimens were available from 120 localities. The analysis of variation of adult ticks was limited to localities from which larval samples were also available to allow comparison of patterns of variation of the three stages. Two additional localities having only adults were added to fill in gaps in distribution of the adult stage. Figure 1 is a map of North America showing distribution and code numbers of the various localities. A more detailed listing of locality information, including locality code number, locality descriptions, hosts, dates of collection, and life history stages represented, is given in Table 1 of Thomas (1967).

b. Preparation of Specimens

Male, female, and larval tick specimens were cleared in lactic acid for about 24 hours at a temperature of 50° C. After rinsing in water, all stages

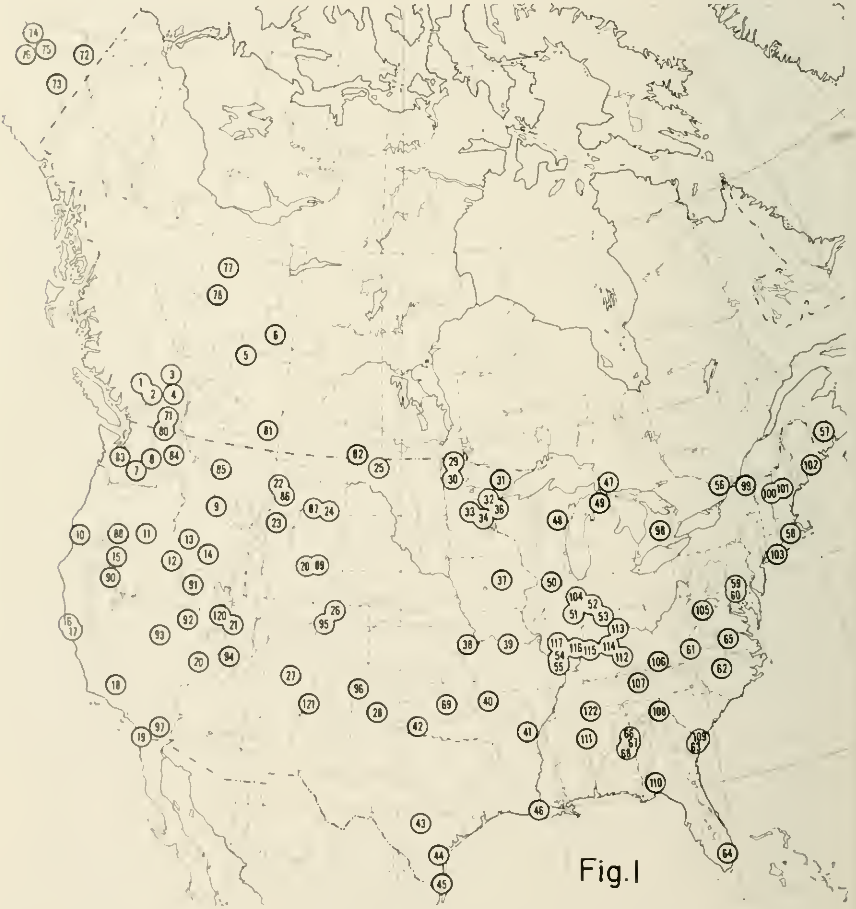

FIG. 1. Map of locality code numbers used in this study. A detailed listing of the localities is given in Table 1 in Thomas (1967). Localities are represented by center of circles. Four of the 122 localities are not plotted: 118 and 119 lacked sufficient locality information and 79 and 35 were indistinguishable from 71 and 36, respectively.

were mounted on microscope slides in Hoyer's mounting medium and dried in an oven for several days. They were ringed with Zutt's ringing compound to prevent deterioration of the mounting medium. Measurements of tick characters were taken using a Reichert microprojector and a millimeter ruler to measure the projected image. In order to randomize day to day errors in measurement, no more than one tick from a given locality was measured on any one day.

c. Characters

Selection of characters for analysis was determined by a pilot study of samples from throughout the range of the tick. Characters lacking variation among localities, such as number of sensilla auriformia, were excluded from the study. The main criterion in character selection was the existence of well defined landmarks to facilitate repeatability of measurement. Non-sclerotized parts subject to distortion by engorgement were avoided. Counts of the number of hypostomal denticles were eliminated because of difficulty encountered in distinguishing the distal elements. Measurements of setal lengths were dropped from the study because of the slope of the setae and the inability to obtain sufficient magnification. The repeatability of a measurement was tested by remeasuring a series of ticks at a later date without reference to the original values obtained. The mean difference between the two sets of measurements obtained was calculated and if this difference was less than implied range of the values given, the variable was considered to be repeatable. Characters were chosen from different areas of the tick, such as the scutum, the appendages, and the capitulum to be as representative of the entire tick as possible. Adult characters homologous to the most important larval characters were chosen to allow comparison of characters between the three stages studied, i.e., males, females, and larvae.

A description of the 16 larval characters studied follows. Character numbers precede the descriptions for each of the characters: (1) Width of scutum measured just behind the sensilla sagittiformia located on the dorsal lateral body surface. (2) Median length of scutum.

In the following measurements of appendages, each segment of the appropriate leg was measured along antero-lateral margin of the right appendage. If the right leg was missing, the left one was measured. If both members of a pair of appendages were missing, the tick was excluded from the analysis. (3) Length of tarsus I. (4) Length of coxa III. (5) Length of trochanter III. (6) Length of basifemur III. (7) Length of telofemur III. (8) Length of genu III. (9) Length of tibia III. (10) Length of basitarsus III. (11) Length of telotarsus III. (12) Greatest width of anal plate. (13) Width of basis capituli measured dorsally along a line through the dorsal sensilla hastiformia on the basis. (14) Combined length of palpal articles II and III measured dorsally along anterolateral margin. (15) Length of hypostome measured from the posthypostomal seta to last well-sclerotized denticle. (16) Length of right chelicera including moveable digit.

The six characters used in the analysis of male and female variation are identical to larval characters 1, 8, 9, 13, 14, and 15 and bear the same numerical designation with the following exceptions. Character 1 of the male is the width of scutum measured just behind the level of the coxa of leg II. Character 1 of the female is the width of scutum measured just behind the level of the coxa of leg I.

d. Computations and Design of Analyses

Statistical computations were carried out on a desk calculator and on IBM 1620 and 7040 digital computers using programs written in FORTRAN II and IV.

Availability of collections of tick specimens from different host individuals, different host species and different localities permitted structuring of statistical analyses to consider variation and covariation at several levels.

1. 64-Locality Study of Larvae.—A preliminary study of 16 characters of larvae from 64 localities was made to obtain an idea of the overall pattern of character variation and covariation within and among localities. Since equal sample sizes simplify computations, only localities having a minimum of 15 ticks per locality were included in this analysis. At each locality the larvae were usually from a single host individual although samples of ticks from several host individuals were used at a few localities to obtain required sample size of 15. The localities used in this study are designated as locality code numbers 1 through 64 in Figure 1.

2. 120-Locality Study of Larvae.—To determine effect of increasing the number of localities sampled on magnitude and stability of variance components, larvae from 56 additional localities were included with the original 64 and the data reanalyzed. These additional localities, containing unequal sample sizes, are given in Figure 1 as locality code numbers 65 to 119 and code number 122. In consideration of evidence to be presented later, concerning variation of characters among different host individuals of the same species within a locality, ticks were randomly chosen from as many collections as were available for each of the 120 localities to obtain a more representative sample.

The number of characters of ticks used in this and subsequent studies was reduced from 16 to six by means of factor analysis of character correlations from the 64-locality study to isolate independent patterns of character variation. This approach was previously used by Sokal and Rinkel (1963) and will be described in more detail below.

3. 38-Locality Study of Male Ticks.—An analysis of variation and covariation of six characters of males was made within and among 38 localities. The six characters used were homologous to those used in previous larval study

and following female study. The samples were variable in size, with a maximum of 15 ticks per locality, and were usually from more than one host individual.

4. 36-Locality Study of Female Ticks.—Females from 36 localities were analyzed for intra- and interlocality variation and covariation. Sample size was variable with a maximum of 15 ticks from one or more host individuals from each locality.

5. 33-Locality Study of Larvae.—A sample of larval ticks from localities having both males and females was analyzed to compare patterns of variation and covariation of larval characters with those of adults. Larval ticks were from one or more host individuals but not necessarily from the same host individual or species as adult samples.

Several other designs, with limited applications, are described in the relevant sections.

SOURCES OF VARIATION

Model II analysis of variance permits partitioning of variation into various levels depending on complexity of the data sampled. This technique has been applied in studies of the gall forming aphid, *Pemphigus populi-transversus*, by Sokal (1952, 1962), Sokal and Rinkel (1963), and Sokal and Thomas (1965). This approach has also been used by Mason (1964) in analysis of geographic variation of a cerambycid beetle, *Tetraopes tetraophthalmus.*

A knowledge of percentages of variation at various levels in the analysis provides insight of the population structure of the species considered and gives clues to action of evolutionary forces. With proper experimental design, genetic and environmental components of variation can be separated. Consideration of the amount of variation at various levels within the analysis is of value in planning future studies. Fewer samples are needed at areas of low variability and more samples from areas of high variability to provide more reliable estimates of parameters to be estimated. In this study, variation was partitioned to determine relative amounts of variation of characters of ticks among localities and within localities, among different host individuals within a locality, among different host species and among different host individuals of the same species. Estimates of relative amounts of variation will be compared not only from character to character but also among different life forms, i.e., larvae, males, and females.

a. 64-Locality Study of Larvae

The structure of this study permitted calculation of variance within and among localities with 896 and 63 degrees of freedom, respectively. Single classification analyses of variance (Model II; Steel and Torrie, 1960) were performed on each of the 16 characters and variance components estimated, All characters had highly significant *F*-values ($P < 0.01$). Table 1 shows percent of total variation of each character attributed to the variance component among localities. The percentage attributed to within-locality variance component (not shown) is the 100-complement of value given. The percentage of variation among localities ranges from 45.67% for length of basitarsus III to 80.86% for length of trochanter III. In general there is a high degree of differentiation among localities for all characters considered.

TABLE 1. Variance Components among Localities Expressed as Percentage of Total Variation (64- and 120-Locality Studies of Larvae).

	Characters and their code numbers	64-locality study of larvae	120-locality study of larvae
(1)	Width of scutum	75.29	74.96
(2)	Length of scutum	62.84	
(3)	Length of tarsus I	62.06	
(4)	Length of coxa III	62.21	
(5)	Length of trochanter III	80.86	
(6)	Length of basifemur III	41.63	
(7)	Length of telofemur III	66.04	
(8)	Length of genu III	53.04	48.50
(9)	Length of tibia III	46.91	44.69
(10)	Length of basitarsus III	45.67	
(11)	Length of telotarsus III	47.33	
(12)	Width of anal plate	60.47	
(13)	Width of basis capituli	69.56	68.67
(14)	Length of palp	77.35	73.92
(15)	Length of hypostome	59.98	56.19
(16)	Length of chelicera	48.49	

Variances were calculated for every character for each locality and Bartlett's test of the homogeneity of variances applied (Snedecor, 1956). Significant X^2 values, indicating unequal variances, were obtained for all characters except length of the scutum. No apparent reason could be found for large variances at certain localities. Plotting of coefficients of variation on maps did not show any geographic pattern of high or low variability. There did not appear to be any relationship between the magnitudes of the means and those of the variances, therefore a transformation did not seem appropriate. Samples of ticks derived from several host individuals from a given locality did not show a greater variance than samples from a single host individual. Known sibships of ticks did not show a smaller variance than those normally found in a wild-caught population. Since equal variances are an assumption of the analysis of variance, another technique (allowing for the occurrence of heterogeneous variance) was used to test the hypothesis that the means considered were from the same population. The method used was one suggested by Snedecor (1956). In this procedure two weighted mean squares are calculated and their ratio is tested against the *F*-distribution. The values obtained indicated significant differences of means for all characters, confirming the results of the analysis of variance. An examination of the frequency distribution of variances for each character showed that they approximated a X^2-distribution with only a few outlying values contributing to the heterogeneity of variances. Therefore, it was concluded that existing heteroscedasticity did not affect results of the analysis of variance. This conclusion is supported by findings of Box (1954) who showed that if groups are equal in a one-way classification analysis of variance, moderate inequality of variance does not seriously affect power of the test.

b. 120-Locality Study of Larvae

Single classification analyses of variance, with 119 and 1,290 degrees of freedom among and within localities, respectively, were carried out for six characters selected as representative of patterns of variation of the 64-locality study by methods to be discussed below. The right hand portion of Table 1 lists the percentages of variation due to the interlocality variance components in the 120-locality study which are comparable in magnitude to those obtained in the 64-locality study. All *F*-ratios were highly significant.

c. 38-Locality Study of Males and 36-Locality Study of Females

Results of single classification analyses of variance for each character of males and females are given in Table 2 as percentage of total variation due to the interlocality variance component. These values are 74.9 to 91.0 in males and 76.4 to 88.1 in females. The percentages are similar in both sexes for a given character. All *F*-ratios were highly significant.

d. 33-Locality Study of Larvae

TABLE 2. Variance Components among Localities Expressed as Percentage of Total Variation (Larvae, Males and Females).

Characters and their code numbers	33-locality study of larvae	38-locality study of males	36-locality study of females
(1) Width of scutum	80.21	77.87	80.42
(8) Length of genu III	53.33	74.88	80.86
(9) Length of tibia III	46.16	77.26	76.36
(13) Width of basis capituli	65.73	91.02	88.14
(14) Length of palp	76.61	85.04	79.43
(15) Length of hypostome	66.55	85.31	81.59

Results of single classification analyses of variance for the six larval characters of this study are shown in the first column of Table 2. All *F*-ratios were highly significant. The interlocality variance components are similar to those of the 64- and 120-locality studies of larval characters in Table 1 but are generally lower than corresponding values for males and females in Table 2. These results suggested a further comparison of variation in larvae, males and females. Variances were corrected relative to the magnitude of the character means. Thus for each character in each of the life history stages, the square root of the error variance component was divided by the grand mean. When multiplied by 100, this gave a coefficient analogous to an average coefficient of variation. A similar operation was carried out for the interlocality variance component. These two coefficients are shown in Table 3 for every character from the 64-locality, 120-locality and 33-locality larval studies, the 38-locality male study, and the 36-locality female study. The interlocality coefficient of variation is almost always greater than the intralocality coefficient. The three studies based on larval ticks are similar in their results, but coefficients from these studies are markedly smaller than those of the males and females within localities and even more so than those among localities.

Coefficients of variation were calculated for each locality and each character of larvae, males and females, from the same 33 localities. From these the

TABLE 3. Intra- and Interlocality Coefficients of Variation.

	Characters and code numbers	64-locality study of larvae	120-locality study of larvae	33-locality study of larvae	38-locality study of males	36-locality study of females
(1)	Width of scutum	2.86 5.00	2.97 5.13	2.80 5.63	4.89 9.17	4.59 9.31
(2)	Length of scutum	3.39 4.41				
(3)	Length of tarsus I	3.15 4.03				
(4)	Length of coxa III	4.20 5.39				
(5)	Length of trochanter III	3.09 6.35				
(6)	Length of basifemur III	6.28 5.30				
(7)	Length of telofemur III	3.34 4.66				
(8)	Length of genu III	3.22 3.53	3.34 3.24	3.18 3.40	4.95 8.55	4.20 8.64
(9)	Length of tibia III	3.87 3.64	3.70 3.32	3.56 3.30	5.03 9.28	4.79 8.60
(10)	Length of basitarsus III	5.18 4.75				
(11)	Length of telotarsus III	4.08 3.87				
(12)	Width of anal plate	5.04 6.24				
(13)	Width of basis capituli	3.08 4.66	3.30 4.88	3.41 4.72	3.55 11.30	3.42 9.33
(14)	Length of palp	3.34 6.18	3.40 5.72	3.38 6.12	3.86 9.20	3.86 7.59
(15)	Length of hypostome	3.84 4.70	3.78 4.28	3.73 5.26	4.80 11.56	4.25 8.95
(16)	Length of chelicera	3.27 3.17				

Explanation: For each character and study ______
the upper left coefficient is $100\ [(\sqrt{\text{error variance component}})\ /\ \text{grand mean}]$;
the lower right coefficient is $100\ [(\sqrt{\text{locality variance component}})\ /\ \text{grand mean}]$

average coefficient of variation for each character of larvae, males and females and its standard error were computed. These values are given in Table 4 and are very similar in magnitude to the intralocality coefficients of Table 3. This table also shows results of three significance tests of differences in coefficients of variation for each character over all localities by the sign test (Siegel, 1956, p. 68). There are no significant differences in mean coefficients of variation between male and female characters. Larvae are significantly less variable than males in all characters but length of hypostome, and less variable than females in all characters except length of palp and length of hypostome.

e. Variation of Larval Characters among Host Individuals of the Same Species within Localities

Separate single classification analyses of variance for six larval characters were calculated for each of 36 collections from 30 different localities having samples of larvae from two or more host individuals of the same host species. The number of host individuals and number of larvae per host individual varied from study to study. The individual host specimens are considered as a random sample of those present at a given locality. This permitted estimation of variance components among host individuals for a given locality shown in Table 5 as percent of total variation together with an indication of their significance. The percentages of variation among host individuals vary from 0 to 82.8%. Some localities show no added variance of larval characters among different host individuals while other localities show additional variation at this level in all characters. No one character seems to be more variable than the others.

TABLE 4. Mean Coefficients of Variation of Characters of Larvae, Males and Females for 33 Localities.

Characters and their code numbers	Larvae	Males	Females	Results of sign test for significant difference of means		
				L vs. ♂	L vs. ♀	♂ vs. ♀
(1) Width of scutum	2.63 ±0.136	4.57 ±0.322	4.53 ±0.235	‡	‡	n.s.
(8) Length of genu III	3.07 ±0.140	4.60 ±0.416	4.12 ±0.244	‡	*	n.s.
(9) Length of tibia III	3.48 ±0.144	4.77 ±0.404	4.90 ±0.319	*	*	n.s.
(13) Width of basis capituli	2.81 ±0.115	3.46 ±0.259	3.51 ±0.237	*	‡	n.s.
(14) Length of palp	3.03 ±0.141	3.90 ±0.217	3.64 ±0.227	†	n.s.	n.s.
(15) Length of hypostome	3.77 ±0.165	4.65 ±0.346	3.91 ±0.319	n.s.	n.s.	n.s.

Explanation: The ± value beneath each coefficient of variation is its standard error. The symbols for results of sign test indicate presence or absence of significant differences in mean coefficient of variation for the given comparison:
n.s. = not significant; * = $0.01 < P \leq 0.05$; † = $0.001 < P \leq 0.01$; ‡ = $P \leq 0.001$.

TABLE 5. Variance Components among Host Individuals Expressed as Percentage of Total Variation.

Locality code no.	Host species and no. of host individuals	Characters and their code numbers					
		Width of scutum (1)	Length of genu III (8)	Length of tibia III (9)	Width of basis capituli (13)	Length of palp (14)	Length of hypostome (15)
3	3 *Lepus americanus*	4.03	36.44‡	23.80*	9.90	3.26	0
8	2 *Sylvilagus nuttallii*	17.04	15.76	0	0	30.43*	37.15‡
9	4 *Sylvilagus nuttallii*	32.13‡	18.32*	17.69*	0	0	9.34
11	3 pigmy rabbits	48.42*	6.66	12.64	42.92*	55.32	30.33
11	3 cottontails	48.01	30.95	13.88	20.31	19.49	31.47
13	15 jackrabbits	14.61*	20.60†	15.35*	5.40	14.63*	21.70‡
16	2 *Sylvilagus bachmani*	4.61	0	2.33	2.49	6.92	0
27	4 cottontails	0	30.69	12.17	0	11.18	0
31	2 ruffed grouse	6.72	0	37.23‡	23.90*	12.60	14.75
32	2 ruffed grouse	0	0	0	0	0	0
33	4 *Sylvilagus floridanus*	31.77‡	11.14*	13.07*	32.37‡	34.88‡	8.35
33	6 snowshoe hares	19.42‡	15.86‡	8.62*	29.39‡	20.59‡	6.87
33	4 ruffed grouse	2.70	31.68‡	34.18‡	14.15†	37.36‡	22.43‡
34	2 ruffed grouse	4.94	15.11	0	64.18‡	49.81‡	27.41*
35	2 ruffed grouse	13.77	0	20.68*	3.86	8.31	0.68
36	2 ruffed grouse	0	25.60*	27.27*	4.33	0	55.51‡
37	6 *Sylvilagus floridanus*	12.18*	14.96†	0	2.15	13.10*	18.02‡
38	7 *Sylvilagus floridanus*	51.62‡	42.83‡	42.14‡	37.33‡	61.35‡	35.90‡
40	16 *Sylvilagus floridanus*	6.09	0	3.82	10.38	3.42	25.62‡
49	2 ruffed grouse	6.28	0	0	0	56.59‡	0
49	3 snowshoe hares	26.13*	55.37‡	59.22‡	0	12.52	25.70*
50	6 *Sylvilagus floridanus*	1.85	11.00*	26.46‡	15.33‡	9.97*	0
52	2 *Sylvilagus floridanus*	5.31	0	0	0	0	0
54	8 *Sylvilagus floridanus*	27.15‡	15.57‡	8.70*	20.87‡	30.18‡	37.00‡
55	(Cobden) 5 cottontails	30.53‡	21.54‡	30.72‡	56.74‡	37.83‡	28.11‡
55	(Ware) 2 cottontails	43.90‡	22.57*	35.21†	36.70‡	60.31‡	74.24‡
57	3 ruffed grouse	9.64	26.09†	0.46	0.30	16.15*	24.04*
58	5 cottontails	0	82.83‡	64.12*	0	63.65*	0
60	4 cottontails	63.16‡	73.43‡	61.72‡	46.11‡	33.83‡	49.34‡
60	7 quail	19.02	17.81	27.61	30.98	0	2.44
62	7 cottontails	0	0	13.37	21.20	0	1.91
64	3 *Sylvilagus palustris*	0	2.52	0	0	45.60†	47.65†
67	3 cottontails	24.22*	0	12.37	31.04*	0	10.56
68	2 bobwhites	5.09	0	0	0	4.25	0
68	2 cottontails	0	0	7.05	56.01†	0	2.95
72	4 tree sparrows	0	0	0	0.10	0	10.69
74	3 sparrow species	6.21	0	3.55	0	24.43	0.90
117	3 *Sylvilagus floridanus*	29.19	0	0	56.01	0	0

Explanation: Locality code numbers refer to localities given in Figure 1. Reference marks indicate the significance of the variance components used to calculate the percentages given in the table.

* = $0.01 < P \leq 0.05$; † = $0.005 < P \leq 0.01$; ‡ = $P \leq 0.005$; no asterisk = not significant.

To test the hypothesis that seasonal and annual variation in tick characters might occur within a given locality, a two-level hierarchic analysis of variance (Steel and Torrie, 1960) was calculated for six characters at each of four different localities. The levels of variation in these four analyses were among years, within years and error. The type of collections required for an analysis of this type were limited, but suitable samples were available from Lee

County, Illinois, Durham County, North Carolina, Washington County, Arkansas, and Cheboygan County, Michigan. Results of these analyses seemed to negate existence of year to year character variation. Larval tick characters 1, 14, and 15 each showed a significant variance component among years at one locality.

f. Variation of Larval Characters among Host Species and among Host Individuals of the Same Host Species

To determine influence of different host species upon variation of larval ticks, separate hierarchic Model II analyses of variance with Satterthwaite's approximation for estimation of variance components when using unequal sample sizes (Ostle, 1954) were calculated for 12 different localities having samples of ticks from different individual hosts as well as different host species. The levels of variation in this analysis were among different host species, among different host individuals within a host species, and error. The number of host species varied from locality to locality, the number of host individuals within a species varied, as did the number of ticks from each host individual within and among the 12 studies. Again six larval characters were used at each locality with exception of a few localities where all 16 characters were measured.

Table 6 gives variance components (and their significance) among host species and among host individuals of the same host species expressed as a percentage of total variation. Roughly half of the localities have significant additional variance among host individuals within host species for each of the characters, whereas at most three localities show additional variation among host species for any given character. Tick samples from localities having only resident host species, i.e., cottontails, jackrabbits and snowshoe hares generally do not show added variance of larval characters among host species. However, larvae from localities having samples from migratory birds do show such added variance.

In comparing means of ticks from migratory birds as contrasted with resident host species, the analysis of variance can be interpreted as a Model I. Twelve of the 16 characters of ticks examined at Tama County, Iowa (Locality Code number 37), showed significant differences among host species. Inspection of means from the different host species showed that ticks from migratory birds showed considerable deviation from ticks from resident host species such as cottontails. A sample of ticks from a slate-colored junco had the highest mean values for all 16 characters. Twelve of these means were highly significantly different from means obtained for ticks from cottontails in this area. However, ticks from the slate-colored junco did not show any significant differences from tick populations from the Minnesota area (Thomas, 1967). In fall of the year, juncos are migrating southward from their nesting areas in northern North America and it is possible that this

TABLE 6. Variance Components among Host Species and among Host Individuals of the Same Species Expressed as Percentages of Total Variation.

		Characters and their code numbers					
Locality code number	Hosts	Width of scutum (1)	Length of genu III (8)	Length of tibia III (9)	Width of basis capituli (13)	Length of palp (14)	Length of hypostome (15)
9	cottontails and grouse	0 43.06†	0 34.62*	0 0	33.81† 0	50.56‡ 0	68.28* 8.18
11	pigmy rabbits and cottontails	0 46.93*	0 18.84	6.01 10.74	0 35.26	14.62 40.49*	6.79 31.09
12	jackrabbits and cottontails	0 0	0 41.90	0 61.52	0 0	23.16 0.93	52.72‡ 0
13	jackrabbits and cottontails	0 15.36*	0 19.93†	0 14.62*	0 7.16	0 13.90*	0 20.53‡
33	snowshoe hares, grouse, cottontails	0 20.80‡	0.61 18.56‡	0 18.11‡	0 26.39‡	2.25 29.51†	0 10.65‡
37	cottontails, many bird species	38.49‡ 4.50*	10.06* 7.84*	10.98* 8.33†	32.57‡ 6.88†	3.24 16.61‡	17.87‡ 6.39*
40	cottontails, few bird species	18.24* 1.82	0 0	0 4.49	0 10.16	0 2.0	0 25.91‡
49	snowshoe hares, grouse	0 23.57*	0 45.70‡	0 47.86‡	7.59 0	0 32.89‡	14.06 10.94
57	snowshoe hares, grouse	0 8.41	0 28.43‡	2.13 0.92	19.46* 0.57	0 16.43*	0 26.12†
60	cottontails and quail	0 54.12‡	0 65.76‡	0 55.40‡	0 41.70‡	28.09* 17.66†	0 40.02‡
67	cottontails and quail	0 25.56*	4.17 0	0 11.93	0 27.98*	0 0	0 11.94
68	cottontails and quail	19.66* 1.39	8.83 0	26.90† 0	0 9.48	5.97 1.36	0 0

Explanation: Locality code numbers refer to localities given in Figure 1. For each character and locality the upper left term is the percentage of total variation among host species; the lower right term is the percentage of total variation among host individuals of the same host species. The percentage of the third level of variation (error) is not given but would be the 100-complement of the sum of the two values given. Reference marks indicate significance of the variance components used to calculate the percentages given in the table.

* = $0.01 < P \leq 0.05$; † = $0.005 < P \leq 0.01$; ‡ = $P \leq 0.005$; no asterisk = not significant.

particular bird picked up its population of ticks in Minnesota and then flew on to Iowa.

g. Variation of Larval Characters among Localities and among Host Individuals within Localities

Model II hierarchic analyses of variance tested whether variation of larval characters among localities was in excess of their variation among host in-

TABLE 7. Variance Components among Localities and among Host Individuals within Localities Expressed as Percentages of Total Variation.

	Characters and their code numbers					
Host species	Width of scutum (1)	Length of genu III (8)	Length of tibia III (9)	Width of basis capituli (13)	Length of palp (14)	Length of hypostome (15)
67-Locality study of cottontails	54.22‡	28.00‡	20.80‡	28.64‡	24.90‡	28.33‡
	12.35‡	15.81‡	17.50‡	16.72‡	4.41‡	18.43‡
32-Locality study of *Sylvilagus floridanus*	41.20‡	8.76*	10.17†	28.18‡	15.53‡	7.56*
	16.40‡	21.81‡	22.06‡	19.41‡	26.23‡	25.11‡
15-Locality study of *Sylvilagus nuttallii*	7.07	9.29	32.44‡	24.05‡	12.14‡	11.72
	27.15‡	12.41*	4.93	1.98	2.83	20.43‡
3-Locality study of *Sylvilagus idahoensis*	0	0	7.43	0	0	42.98
	37.34*	10.20	22.46	39.59*	50.65†	8.63
2-Locality study of *Sylvilagus palustris*	55.46‡	40.10	9.35	32.42‡	64.57	18.23
	0	3.54	0	0	15.10‡	42.37‡
3-Locality study of *Sylvilagus aquaticus*	0	0	0	27.97	17.81	54.97
	75.76‡	33.26*	36.10*	1.41	0	2.83
15-Locality study of ruffed grouse	12.54‡	3.76	0	17.28	13.17	17.71
	3.37	17.60‡	18.33‡	14.71‡	21.94‡	20.30‡
21-Locality study of snowshoe hares	31.29*	6.71	2.76	37.01*	35.11*	35.72‡
	11.55‡	28.56‡	26.39‡	11.44‡	8.62‡	4.08
11-Locality study of jackrabbits	54.91‡	27.30	11.56	36.69†	46.66‡	39.97*
	7.28†	24.15‡	22.22‡	11.64‡	14.78‡	19.98‡
7-Locality study of bobwhite quail	9.87*	8.09	21.05‡	0	0	18.61‡
	7.37	.97	6.22	12.53	0	0

Explanation: For each character and host species the upper left term is the percentage of total variation among localities; the lower right term is the percentage of total variation among host individuals within localities. The percentage of total variation of the third level (error) is not given but is the 100-complement of the sum of the two values given. Reference marks indicate the significance of the variance components used to calculate the percentages given in the table. * = $0.01 < P \leq 0.05$; † = $0.005 < P \leq 0.01$; ‡ = $P \leq 0.005$; no asterisk = not significant.

dividuals within localities. Samples of ticks from nine different host species were analyzed separately for a number of localities. The number of localities used varied among host species. The number of host individuals per locality and the number of ticks per host individual were variable within each host species.

Table 7 gives variance components among locality and within locality expressed as percentages of total variation as well as an indication of their significance. Inspection of the table reveals that larvae from host species having a wide geographic distribution have significant variation among localities in excess of variation among larvae from different host individuals.

h. Section Summary

Larval characters exhibited variance components among host individuals within a locality in excess of that among larvae within a host. The amounts of such added variance differed from character to character and from locality to locality without any regular pattern. The characters showed no seasonal variability. No added variance of larval characters occurred among resident host species, but larvae collected on non-resident species (migratory birds) showed significant differences in many characters from larvae on resident host species, suggesting that migratory birds play a role in tick dispersal. Interlocality variation of all larval characters was found to be significantly greater than variation of these characters among individuals of host species having a wide geographic distribution.

Male and female characters showed greater variability than homologous larval characters within and among localities.

DESCRIPTION AND ANALYSIS OF COVARIATION

An analysis of covariation of characters is of interest for a variety of reasons. Covariation is often indicative of coadaptation of characters to common selective forces or of the presence of common genetic causal factors. A knowledge of patterns of covariation allows the research worker to avoid duplicating information already obtained by considering only one or two of the correlated characters, therefore preventing redundancy in the study. The correlation (concordance) of characters over localities is of considerable interest for attempts at the definition of subspecies.

In this study, it is possible not only to consider covariation of characters within a life history stage, but available data also allow comparisons among larvae, males and females. This is only the second study of congruence of geographic variation of two or more morphotypes of a species known to the author. The other example is that of Sokal and Thomas (1965) on covariation of stem mothers and alates of the aphid *Pemphigus populi-transversus.*

a. Correlation Coefficients

The structure of the data allowed computation of character correlations within as well as among localities. The correlations were computed as product-moment coefficients at the intralocality level and as product-moment and component coefficients at the interlocality level (Sokal, 1962). These three types of correlation matrices were obtained for the 33- and 64-locality studies of larval characters, the 38-locality study of male characters and the 36-locality study of female characters, allowing for comparison of patterns of covariation in these studies.

Table 8 contains intralocality correlations from the 64-locality study of larvae. The coefficients are relatively small in magnitude but all are significant at $P \leq 0.01$ except for several correlations of length of basifemur III with other characters.

A cluster analysis (Fruchter, 1954) of the intralocality correlation matrix produced one large cluster of correlations of appendage characters (character numbers 3, 5, 8, 9, 10, and 11) with width of basis capituli and length of palp. The length and width of scutum and width of coxa III form another cluster connected to the first one at a lower level of correlation. The length of basifemur III, width of anal plate and length of hypostome appear to represent three independent trends of variation in this correlation matrix as they do not cluster with each other or any of the other variables.

TABLE 8. Intralocality Product-moment Correlations of Characters of Larvae (64-Locality Study).

Character code numbers	Characters and their code numbers															
	Width of scutum (1)	Length of scutum (2)	Length of tarsus I (3)	Length of coxa III (4)	Length of trochanter III (5)	Length of basifemur III (6)	Length of telofemur III (7)	Length of genu III (8)	Length of tibia III (9)	Length of basitarsus III (10)	Length of telotarsus III (11)	Width of anal plate (12)	Width of basis capituli (13)	Length of palp (14)	Length of hypostome (15)	Length of chelicera (16)
(1)	X	46	37	46	45	14	39	38	33	30	33	27	47	40	27	32
(2)		X	31	40	31	11	33	33	31	28	28	22	35	41	26	39
(3)			X	39	47	15	49	54	48	44	59	24	46	51	25	38
(4)				X	33	*04*	36	44	39	37	39	26	33	36	28	33
(5)					X	28	60	52	39	28	34	28	38	46	31	35
(6)						X	22	*03*	*–01*	–09	*–06*	*08*	10	20	13	14
(7)							X	65	48	32	43	22	39	50	34	34
(8)								X	70	52	62	28	42	48	33	35
(9)									X	66	59	23	30	42	30	32
(10)										X	61	24	31	32	24	26
(11)											X	20	36	39	23	29
(12)												X	23	27	22	25
(13)													X	51	23	24
(14)														X	35	45
(15)															X	30
(16)																X

Explanation: All coefficients not significant at P ≦ 0.01 are in italics ($r \leqq 0.08$). Decimal points have been omitted.

Table 9 shows the interlocality correlations from the same study with component correlations above the diagonal and product-moment correlations below the diagonal. Inspection of the two half-matrices shows that magnitudes and pattern of the coefficients are similar to each other. All product-moment coefficients are significant at P ≦ 0.01 except for the correlation of width of scutum with several characters. Significance tests for component correlations are not known; the significance levels of the product-moment matrix of coefficients are used as an approximation.

TABLE 9. Interlocality Product-moment and Component Correlations of Characters of Larvae (64-Locality Study).

	Characters and their code numbers															
Character code numbers	Width of scutum (1)	Length of scutum (2)	Length of tarsus I (3)	Length of coxa III (4)	Length of trochanter III (5)	Length of basifemur III (6)	Length of telofemur III (7)	Length of genu III (8)	Length of tibia III (9)	Length of basitarsus III (10)	Length of telotarsus III (11)	Width of anal plate (12)	Width of basis capituli (13)	Length of palp (14)	Length of hypostome (15)	Length of chelicera (16)
(1)	X	82	60	88	64	*31*	75	69	70	75	72	81	76	*30*	26	46
(2)	80	X	77	80	87	59	85	67	77	82	75	86	77	60	62	74
(3)	59	76	X	78	90	59	82	68	84	88	91	63	81	78	62	79
(4)	87	79	77	X	79	49	89	80	82	81	83	78	80	60	47	66
(5)	63	86	89	77	X	72	90	63	79	83	74	78	81	84	75	80
(6)	*30*	56	56	46	69	X	72	43	52	48	42	52	49	78	75	67
(7)	74	83	81	87	89	68	X	86	90	88	80	82	72	76	66	68
(8)	68	65	67	78	62	40	85	X	92	82	76	66	47	45	41	41
(9)	68	75	82	80	77	48	88	91	X	92	84	75	66	64	56	60
(10)	72	79	85	78	80	43	85	80	90	X	92	80	75	62	51	62
(11)	70	73	89	81	72	38	78	75	82	89	X	61	75	59	39	67
(12)	80	84	62	76	77	49	80	64	72	76	59	X	75	51	53	59
(13)	76	75	80	79	80	47	71	46	64	72	73	74	X	60	49	77
(14)	*30*	60	77	60	84	74	75	45	62	61	58	50	60	X	78	79
(15)	*26*	61	60	46	74	71	65	41	54	50	38	52	48	76	X	74
(16)	45	72	76	64	77	63	66	41	58	60	64	57	74	77	71	X

Explanation: Interlocality component correlations are above diagonal; interlocality product-moment correlations are below diagonal. All coefficients not significant at $P \leq 0.01$ are in italics ($r \leq 0.32$). Since significance levels of component correlations are not known the significance levels of product-moment correlations with 62 degrees of freedom have been applied. Decimal points have been omitted.

Cluster analyses of the two matrices confirm their similarity. Three distinct clusters containing the same variables appeared. The first cluster is composed of variables representing general body size such as length and width of scutum, length of coxa III, width of anal plate, and width of basis capituli. This cluster also contains two appendage characters, length of trochanter III and length of telofemur III. The second cluster is made up entirely of appendage characters including length of tarsus I, length of genu III, length of tibia III, length of basistarsus III, and length of telotarsus III. The third cluster contains length of palp, length of hypostome, and length of chelicera, as well as length of basifemur III.

Correlations were computed for only six characters in the 33-locality study of larvae, 38-locality study of males and 36-locality study of females. These matrices are given in Tables 10 and 11. All correlation coefficients in the three intralocality correlation matrices (Table 10) are significant at $P \leqq 0.01$.

A cluster analysis of larval intralocality coefficients $\geqq 0.50$ showed three groups. The two most highly correlated variables are length of genu III and

TABLE 10. Intralocality Product-moment Correlations of Characters of Larvae, Males and Females.

	Character code number	Characters and their code numbers					
		Width of scutum (1)	Length of genu III (8)	Length of tibia III (9)	Width of basis capituli (13)	Length of palp (14)	Length of hypostome (15)
	(1)	X	44	42	51	48	28
	(8)		X	69	43	49	35
Larvae	(9)			X	34	45	32
n = 440	(13)				X	56	23
	(14)					X	32
	(15)						X
		(1)	(8)	(9)	(13)	(14)	(15)
	(1)	X	57	52	58	39	33
	(8)		X	77	61	57	53
Males	(9)			X	57	48	45
n = 440	(13)				X	59	50
	(14)					X	55
	(15)						X
		(1)	(8)	(9)	(13)	(14)	(15)
	(1)	X	41	31	46	31	21
	(8)		X	54	54	57	42
Females	(9)			X	42	39	29
n = 326	(13)				X	60	41
	(14)					X	57
	(15)						X

Explanation: All correlation coefficients are significant at $P < 0.01$. Decimal points have been omitted. *n* is the number of specimens on which each correlation coefficient is based.

length of tibia III. Another cluster contains width of scutum, width of basis capituli, and length of palp. The sixth variable in the study, length of hypostome, does not cluster with the others.

The male intralocality coefficients $\geqq 0.57$ yielded a heterogeneous cluster of five characters and one independent character (length of hypostome). The width of basis capituli and length of genu III form the nucleus of the large cluster. Three other characters (length of tibia III, length of palp and width of scutum) cluster with both of the previously mentioned characters but not with each other.

A cluster analysis of the female intralocality correlation matrix revealed a pattern more closely related to that of the larvae. There is a cluster composed of width of basis capituli and length of palp and a related cluster containing length of genu III and length of tibia III. The length of hypostome is also related to the first mentioned cluster. The width of scutum is independent of the other variables.

The interlocality product-moment and component correlation matrices (Table 11) are very similar to each other for each life history stage and are generally higher than the intralocality correlations of Table 10. All coefficients but one were significant at $P \leqq 0.01$.

Cluster analysis of larval component correlations $\geq$ 0.80 yielded three clusters. The first comprises width of scutum and width of the basis capituli; the second, length of genu III and length of tibia III; and the third, length of palp and length of hypostome.

Male component correlations show two closely related clusters, the first composed of width of scutum, length of genu III and length of tibia III, the other of width of basis capituli, length of palp, and length of hypostome.

Female component correlations clustered somewhat differently. The first cluster includes width of scutum, length of genu III, length of tibia III, and width of basis capituli; the second is closely related to the first and contains length of palp and length of hypostome.

To compare the structure of correlation matrices within and among life history stages, the corresponding elements of two matrices were paired and correlations between them computed using Spearman's rank correlation coefficient (r_s; Siegel, 1956, p. 202). The correlation coefficients in these matrices are not independent, making it difficult to determine appropriate sample size for testing significance of r_s. As a conservative estimate, $n = 6$ (the number

TABLE 11. Interlocality Product-moment and Component Correlations of Characters of Larvae, Males and Females.

	Character code numbers	Width of scutum (1)	Length of genu III (8)	Length of tibia III (9)	Width of basis capituli (13)	Length of palp (14)	Length of hypostome (15)
		Characters and their code numbers					
	(1)	X	75	73	88	*33*	48
	(8)	74	X	92	57	54	52
Larvae	(9)	71	90	X	66	61	58
$n = 33$	(13)	87	56	64	X	48	55
	(14)	*34*	54	60	48	X	83
	(15)	47	52	57	54	81	X
		(1)	(8)	(9)	(13)	(14)	(15)
	(1)	X	93	93	87	72	81
	(8)	92	X	99	75	67	81
Males	(9)	92	98	X	75	68	80
$n = 38$	(13)	86	75	74	X	92	94
	(14)	72	67	68	91	X	91
	(15)	80	80	79	93	91	X
		(1)	(8)	(9)	(13)	(14)	(15)
	(1)	X	95	89	97	79	75
	(8)	94	X	95	89	69	67
Females	(9)	88	94	X	86	70	67
$n = 36$	(13)	96	88	85	X	87	81
	(14)	78	69	69	87	X	92
	(15)	74	66	66	80	91	X

Explanation: Interlocality component correlations are above diagonals; interlocality product-moment correlations are below diagonals. Coefficients not significant at $P \leq 0.01$ are in italics. Since significance levels of component correlations are not known the significance levels of product-moment correlations with the same degrees of freedom have been applied. Decimal points have been omitted. n is the number of replicates used in computing each correlation coefficient.

of characters) can be applied, a less conservative estimate would use the actual number of coefficients compared, $n = 15$. Two probability values, P_1 and P_2, are used below to correspond with these to provide bounds for probability values of the test.

The Spearman rank correlation coefficients between interlocality component and product-moment correlations within a life history stage were all equal to 0.99 ($P_1, P_2 < 0.01$). For this reason only component correlations will be used in the following comparisons of matrices of different life history stages. Comparing intralocality matrices among life history stages, the highest r_s obtained (0.60; $P_1 > 0.05$, $P_2 < 0.05$) is between larval and male matrices. Male against female and larval against female matrices produced essentially identical results with r_s equal to 0.47 and 0.48 respectively ($P_1 > 0.05$, $P_2 < 0.05$).

Comparisons between interlocality component matrices yielded different patterns. The correlation between larval and male matrices is not significant ($r_s = 0.43$; $P_1, P_2 > 0.05$), while correlation between larval and female matrices is significant ($r_s = 0.87$; $P_1 < 0.05$, $P_2 < 0.01$), and that between male and female matrices is less clearly significant ($r_s = 0.59$; $P_1 > 0.05$, $P_2 < 0.05$).

b. Factor Analyses

Factor analysis represents covariation by finding fewer dimensions of variation than the number of variables in a correlation matrix. These new dimensions (factors) not only provide parsimony of description but may represent important biological constructs. Sokal (1952, 1965) has given brief explanations and accounts of the applications of factor analysis to biological problems. The reader is referred to books of Cattell (1952) and Harman (1960) for more detailed accounts of principles and procedures of this method.

Principal axis factor analysis, using Hotelling's method (Harman, 1960), was applied to intralocality product-moment and interlocality component correlation matrices from the 64- and 33-locality studies of larval characters, the 38-locality study of male characters and the 36-locality study of female characters. The decision to use interlocality component correlations instead of interlocality product-moment correlations was somewhat arbitrary in this study because of the great similarity of the matrices. The component correlations should give a better indication of the correlational pattern at the interlocality level because intralocality contributions to the variation and covariation have been removed in the computations.

Various criteria were used for determining completeness of factor extraction. These methods included cluster analysis of character correlations, Rohlf's graphic method of plotting factor number against cumulative sum of the eigenvalues (Rohlf, 1962), Kaiser's criterion (p. 363 in Harman, 1960) of number of eigenvalues greater than 1 when unity is used in diagonals of the correlation matrix, and inspection of residual matrices after extraction of the given number of factors. These techniques did not yield a unique solution for the number of factors to be extracted, but they provided estimates of the probable number which led to a compromise solution. The initial factor extraction was made using unities in the diagonals of the correlation matrices. Once an estimate of the number of factors was obtained the matrices were reanalyzed and estimates of the communalities were iterated until they stabilized within an absolute difference of 0.05.

The principal axis factor loadings were rotated to a non-orthogonal system of coordinates using an analytical method developed by Sokal (1958), called MTAM for mass modification of Thurston's analytical method for rotation to simple structure. Simple structure is achieved by rotation of the factor axes to non-orthogonal positions so that some variables are highly loaded on each factor and each variable is highly loaded on a few but not all factors. Some authors question the validity of rotation to simple structure, but it has yielded meaningful results when applied here and in studies by Stroud (1953), Sokal, Daly, and Rohlf (1961), Sokal (1962), and Sokal and Thomas (1965).

The simple structure solution was converted to a primary pattern matrix of pattern coefficients (Harman, 1960), representing the standard partial regression coefficients of each character on each factor.

Matrices of primary patterns and correlations among factors for intralocality product-moment and interlocality component correlations of the 64-locality study of larvae are given in Tables 12 and 13 respectively. The factor loadings of characters on each factor are also shown as a pattern of pluses and minuses to simplify interpretation of these matrices. The communalities (h^2), the amount of variation of each character explained by common factors, are also given.

Four factors were extracted from the intralocality product-moment correlation matrix (Table 12). Factor I is a body size factor affecting length and width of scutum and length of coxa III. The length of tarsus I and tarsus III make factor II a tarsal factor. Factors III and IV also have their largest factor scores for appendage characters, factor III representing a basal appendage factor of leg III (length of trochanter III, basifemur III, and telofemur III), while factor IV represents a distal appendage factor of leg III (length of genu III, tibia III, basitarsus III and telotarsus III). The low communalities of characters 6, 12, and 15 suggest that they represent independent dimensions of variation, as was previously indicated by cluster analysis.

The primary pattern matrix of interlocality component correlations (Table 13) has three factors similar in some respects to the intralocality factors. Interlocality factor I has high scores for length and width of scutum and width of basis capituli similar to intralocality factor I. Factor II appears to represent a

TABLE 12. Primary Patterns and Correlations among Factors Based on Intralocality Product-moment Correlations of Characters of Larvae (64-Locality Study).

Characters and their code numbers	I	II	III	IV	h^2	I	II	III	IV
(1) Width of scutum	68	04	03	−06	47	+++			
(2) Length of scutum	74	−06	−07	−03	42	+++			
(3) Length of tarsus I	−01	51	18	30	59		++		
(4) Length of coxa III	56	−01	−12	22	40	++			
(5) Length of trochanter III	01	12	64	07	55			++	
(6) Length of basifemur III	−02	01	66	27	28			+++	+
(7) Length of telofemur III	−15	16	65	27	63			+++	+
(8) Length of genu III	−14	17	25	70	73			+	+++
(9) Length of tibia III	−06	06	−02	87	70				++++
(10) Length of basitarsus	13	14	−35	80	59			−	+++
(11) Length of telotarsus III	−07	43	−19	73	67		+		+++
(12) Width of anal plate	35	−05	06	07	18	+			
(13) Width of basis capituli	42	39	04	−06	48	+	+		
(14) Length of palp	32	25	27	02	52	+	+	+	
(15) Length of hypostome	27	−10	22	11	25	+			
(16) Length of chelicera	46	06	11	00	33	+			

Factors	I	II	III	IV
I	X	49	71	65
II	49	X	35	33
III	71	35	X	59
IV	65	33	59	X

Explanation: The upper matrix gives the magnitude of the primary pattern coefficients. The communality (h^2) is the percentage of variation due to common factors for each character. At the right is an abstract of the more important primary pattern coefficients. Plus or minus signs represent the following magnitudes of coefficients (positive or negative, respectively): 4 signs ≥ /0.85/; 3 signs ≥ /0.65/; 2 signs ≥ /0.45/; 1 sign ≥ /0.25/. The lower matrix gives the correlations among the four factors. Decimal points have been omitted.

combination of intralocality factors II, III, and IV, its larger factor coefficients being for leg segments. Interlocality factor III is different from any factors found in the previous analysis and has its principal loadings on characters of the capitulum, i.e., length of palp, length of hypostome, and length of chelicera.

The original correlations can be reconstituted as the product of the primary pattern matrix times the matrix of factor correlations times the transpose of the primary pattern matrix. The mean of absolute differences between the recalculated and original interlocality component correlation coefficients was 0.0315 ± 0.0036 and is indicative of completeness of factor extraction as well as accuracy of the computations.

Results of interlocality factor analysis were used to reduce the number of larval characters for subsequent analyses by selecting characters representative of independent trends of variation (i.e., the three factors). The two characters having the highest loading on each of the three factors in Table 13 were chosen for subsequent analyses. These characters are width of scutum and basis capituli for factor I, length of genu III and tibia III for factor II and length of palp and hypostome for factor III.

TABLE 13. Primary Patterns and Correlations among Factors Based on Interlocality Component Correlations of Characters of Larvae (64-Locality Study).

Characters and their code numbers	I	II	III	h^2	I	II	III
(1) Width of scutum	89	37	–36	90	+++		
(2) Length of scutum	59	21	26	86	+		
(3) Length of tarsus I	16	43	47	86			+
(4) Length of coxa III	55	48	02	88	+	+	
(5) Length of trochanter III	27	17	64	93			++
(6) Length of basifemur III	–14	00	92	69			++++
(7) Length of telofemur III	17	57	36	94		+	
(8) Length of genu III	01	99	–10	88		++++	
(9) Length of tibia III	06	84	14	95		+++	
(10) Length of basitarsus III	29	68	09	91		++	
(11) Length of telotarsus III	31	66	04	82		++	
(12) Width of anal plate	66	23	09	78	++		
(13) Width of basis capituli	72	00	25	82	++		
(14) Length of palp	–20	10	99	87			++++
(15) Length of hypostome	–15	–04	97	74			++++
(16) Length of chelicera	25	–09	77	78			+++

Factors	I	II	III
I	X	61	64
II	61	X	61
III	64	61	X

Explanation: The upper matrix gives the magnitude of the primary pattern coefficients. The communality (h^2) is the percentage of variation due to common factors for each character. At the right is an abstract of the more important primary pattern coefficients. Plus signs represent the following magnitudes of coefficients: 4 signs $\geq$ 0.90; 3 signs $\geq$ 0.75; 2 signs $\geq$ 0.60; 1 sign $\geq$ 0.45. The lower matrix gives the correlations among the three factors. Decimal points have been omitted.

The intralocality product-moment and interlocality component correlation matrices of the 33-locality study of larval characters, the 38-locality study of male characters, and the 36-locality study of female characters were factor analyzed using computational procedures and criteria for estimation of number of factors previously described. The results are given in Tables 14, 15 and 16, respectively.

The three intralocality matrices are very similar in characters most highly loaded on each factor. Factor I has its highest score for width of scutum and factor II strongly influences length of tibia III in all three stages. Factor III affects width of basis capituli and length of palp in all three stages but the loadings of the basis capituli are much higher for larvae and females than for males. Factor IV has its highest score for the hypostome in all three matrices. These results differ from primary pattern loadings based on intralocality product-moment correlations for the same six characters of the 64-locality study of larvae (Table 12). Factor I of the 64-locality study corresponds to two correlated ($r = 0.64$) factors, factors I and III, in the 33-locality study. Factor II of the 64-locality study is similar to factor III of the 33-locality study. Factors III and IV of the 64-locality study ($r = 0.59$) are represented by factor III in the 33-locality study. Factor IV of the 33-locality study does not appear

TABLE 14. Primary Patterns and Correlations among Factors Based on Intralocality Product-moment and Interlocality Component Correlations of Characters of Larvae (33-Locality Study).

Characters and their code numbers	I	II	III	IV	h^2	I	II	III	IV
	Intralocality product-moment								
(1) Width of scutum	98	01	01	00	97	++++			
(8) Length of genu III	–02	71	17	02	68		++		
(9) Length of tibia III	02	93	–08	–02	78		+++		
(13) Width of basis capituli	03	–11	86	–04	64			+++	
(14) Length of palp	–03	11	69	04	58			++	
(15) Length of hypostome	00	01	00	1.00	99				++++
Factors	I	II	III	IV					
I	X	48	64	28					
II	48	X	62	41					
III	64	62	X	36					
IV	28	41	36	X					

Characters and their code numbers	I	II	III	h^2	I	II	III
	Interlocality component						
(1) Width of scutum	80	29	–13	90	+++		
(8) Length of genu III	–03	98	–02	92		++++	
(9) Length of tibia III	07	82	12	88		+++	
(13) Width of basis capituli	95	–12	17	92	++++		
(14) Length of palp	12	10	95	91			++++
(15) Length of hypostome	15	–07	90	87			+++
Factors	I	II	III				
I	X	64	46				
II	64	X	57				
III	46	57	X				

Explanation: For each type of correlation coefficient the upper matrix gives the magnitude of the primary pattern coefficients. The communality (h^2) is the percentage of variation due to common factors for each character. At the right of each primary pattern matrix is an abstract of its more important coefficients. Plus signs represent the following magnitudes of coefficients: 4 signs ≥ 0.95; 3 signs ≥ 0.75; 2 signs ≥ 0.55; 1 sign ≥ 0.35. Beneath each primary pattern matrix are the correlations among its factors. Decimal points have been omitted except where the value is equal to or greater than one.

to be a common factor as it only has appreciable loadings for a single variable, length of hypostome. The independent nature of this variable was suggested in the 64-locality study by its low communality ($h^2 = 0.25$).

The results of factor analyses of interlocality component correlations of larvae, males and females are also given in Tables 14, 15 and 16. The primary pattern matrix of larvae (Table 14) is identical to that obtained in the 64-locality study (Table 13). The primary pattern matrices of male and female characters have only two factors which differ from each other and the larval primary pattern matrix. Factor I of the primary pattern matrix of characters of males is highly loaded by characters 1, 8, and 9 and factor II has high loadings from characters 13, 14, and 15. The primary pattern matrix of female characters is more similar to the primary pattern matrix of larvae than to the

TABLE 15. Primary Patterns and Correlations among Factors Based on Intralocality Product-moment and Interlocality Component Correlations of Characters of Males (38-Locality Study).

Characters and their code numbers	I	II	III	IV	h^2	I	II	III	IV
Intralocality product-moment									
(1) Width of scutum	99	−04	00	−02	91	++++			
(8) Length of genu III	06	69	14	06	75		++		
(9) Length of tibia III	−09	1.01	00	−05	86		++++		
(13) Width of basis capituli	30	19	35	07	58			+	
(14) Length of palp	−06	−04	1.02	−04	89			++++	
(15) Length of hypostome	−02	−01	00	1.01	1.00				++++

Factors	I	II	III	IV
I	X	68	52	40
II	68	X	63	54
III	52	63	X	63
IV	40	54	63	X

Characters and their code numbers	I	II	h^2	I	II
Interlocality component					
(1) Width of scutum	79	21	92	+++	
(8) Length of genu III	1.01	−02	98	++++	
(9) Length of tibia III	1.01	−02	98	++++	
(13) Width of basis capituli	10	89	95		+++
(14) Length of palp	−12	1.06	94		++++
(15) Length of hypostome	19	82	93		+++

Factors	I	II
I	X	76
II	76	X

Explanation: For each type of correlation coefficient the upper matrix gives the magnitude of the primary pattern coefficients. The communality (h^2) is the percentage of variation due to common factors for each character. At the right of each primary pattern matrix is an abstract of its more important coefficients. Plus signs represent the following magnitudes of coefficients: 4 signs ≥ 0.95; 3 signs ≥ 0.75; 2 signs ≥ 0.55; 1 sign ≥ 0.35. Beneath each primary pattern matrix are the correlations among its factors. Decimal points have been omitted except where the value is equal to or greater than one.

male primary pattern matrix. It has high scores for characters 1, 8, 9, and 13 in factor I which represents a combination of factors I and II of the larvae. High coefficients for characters 14 and 15 in factor II correspond to factor III of the larvae.

c. Concordance of Patterns of Variation of Larvae, Males and Females

The covariation among six homologous characters of larvae, males and females was investigated by correlation of mean values for each of these characters (Table 17) over the 33 common localities, producing an 18 × 18 matrix of coefficients with 31 degrees of freedom. To compensate for differences in sample sizes of larval, male and female means, a weighted correlation coefficient was computed by the following formula:

$$r = \sum^{a} n_i(X_i-\bar{X})m_i(Y-\bar{Y}) / \sqrt{\sum^{a}[n_i(X-\bar{X})]^2} \sqrt{\sum^{a}[m_i(Y-\bar{Y})]^2}$$

TABLE 16. Primary Patterns and Correlations among Factors Based on Intralocality Product-moment and Interlocality Component Correlations of Characters of Females (36-Locality Study).

Characters and their code numbers	I	II	III	IV	h^2	I	II	III	IV
Intralocality product-moment									
(1) Width of scutum	1.00	–01	–02	01	98	++++			
(8) Length of genu III	06	26	49	05	54			+	
(9) Length of tibia III	–02	99	–06	–01	91		++++		
(13) Width of basis capituli	07	–07	88	–10	68			+++	
(14) Length of palp	–09	–05	78	16	66			+++	
(15) Length of hypostome	02	–01	06	94	95				+++

Factors	I	II	III	IV
I	X	38	52	18
II	38	X	62	32
III	52	62	X	55
IV	18	32	55	X

Characters and their code numbers	I	II	h^2	I	II
Interlocality component					
(1) Width of scutum	85	16	96	+++	
(8) Length of genu III	1.06	–11	97	++++	
(9) Length of tibia III	99	–06	91	++++	
(13) Width of basis capituli	65	38	94	++	+
(14) Length of palp	06	93	95		++++
(15) Length of hypostome	–01	96	92		+++

Factors	I	II
I	X	75
II	75	X

Explanation: For each type of correlation coefficient the upper matrix gives the magnitude of the primary pattern coefficients. The communality (h^2) is the percentage of variation due to common factors for each character. At the right of each primary pattern matrix is an abstract of its more important coefficients. Plus signs represent the following magnitudes of coefficients: 4 signs $\geq$ 0.95; 3 signs $\geq$ 0.75; 2 signs $\geq$ 0.55; 1 sign $\geq$ 0.35. Beneath each primary pattern matrix are the correlations among its factors. Decimal points have been omitted except where the value is equal to or greater than one.

where a is the number of localities, n_i is the sample size from the ith locality for character X and m_i is the sample size from the ith locality for character Y.

The correlations of characters within a life history stage are all significant except for width of scutum and length of palp of larvae. The intercorrelations of male and female characters are all significant. Width of scutum, length of genu III and tibia III of larvae are significantly correlated with all male and female characters considered. The width of hypostome of larvae is significantly correlated with all adult characters except length of genu III and tibia III of males. The length of palp in larvae has the smallest number of significant correlations with adult characters. It is correlated with palp length of males and all female characters except scutum width and genu III length. The length of hypostome of larvae is correlated with all adult characters except width of scutum, and length of genu III and tibia III of males.

TABLE 17. Weghted Interlocality Correlations of Characters of Larvae, Males, and Females.

Characters and code numbers	(1L)	(8L)	(9L)	(13L)	(14L)	(15L)	(1♂)	(8♂)	(9♂)	(13♂)	(14♂)	(15♂)	(1♀)	(8♀)	(9♀)	(13♀)	(14♀)	(15♀)
Width of scutum of larva (1L)	X																	
Length of genu III of larva (8L)	74	X																
Length of tibia III of larva (9L)	71	90	X															
Width of basis capituli of larva (13L) ..	87	57	64	X														
Length of palp of larva (14L) ..	*35*	55	61	49	X													
Length of hypostome of larva (15L) ..	49	53	58	55	82	X												
Width of scutum of male (1♂)	79	62	56	63	*24*	*39*	X											
Length of genu III of male (8♂)	64	64	54	*42*	*28*	*38*	92	X										
Length of tibia III of male (9♂)	61	61	52	*42*	*31*	*43*	92	97	X									
Width of basis capituli of male (13♂) ..	88	54	54	78	*27*	51	88	77	76	X								
Length of palp of male (14♂) ..	79	55	58	78	54	71	78	73	73	93	X							
Length of hypostome of male (15♂) ..	78	50	46	67	*28*	53	81	78	75	95	93	X						
Width of scutum of female (1♀)	82	65	65	75	*42*	66	86	76	76	89	87	82	X					
Length of genu III of female (8♀)	74	73	68	60	*42*	61	87	85	84	81	80	76	95	X				
Length of tibia III of female (9♀)	62	64	60	53	46	64	80	80	83	75	77	70	88	93	X			
Width of basis capituli of female (13♀)	79	65	64	73	50	72	81	73	73	87	88	80	97	94	90	X		
Length of palp of female (14♀)	63	64	73	66	76	83	62	61	61	69	83	66	83	81	79	89	X	
Length of hypostome of female (15♀)	58	60	64	57	68	81	54	57	57	65	81	72	77	75	71	82	92	X

Explanation: All coefficients not significant at $P \leq 0.01$ are in italics ($r \leq 0.44$). Decimal points have been omitted.

TABLE 18. Primary Patterns and Correlations among Factors Based on Weighted Interlocality Correlations of Characters of Larvae, Males, and Females.

Characters and their code numbers		I	II	III	IV	h^2	I	II	III	IV
(1L)	Width of scutum of larva	91	39	–22	14	96	+++			
(8L)	Length of genu III of larva	18	75	00	34	92		++		
(9L)	Length of tibia III of larva	30	73	12	12	90		++		
(13L)	Width of basis capituli of larva	1.00	26	04	–22	88	++++			
(14L)	Length of palp of larva	–09	23	89	–23	79			+++	
(15L)	Length of hypostome of larva	–02	00	98	–08	86			+++	
(1♂)	Width of scutum of male	20	11	–11	86	95				+++
(8♂)	Length of genu III of male	–21	09	04	1.06	94				++++
(9♂)	Length of tibia III of male	–27	04	12	1.06	92				++++
(13♂)	Width of basis capituli of male	62	–07	04	44	99	++			+
(14♂)	Length of palp of male	41	–13	47	29	95	+		+	
(15♂)	Length of hypostome of male	43	–17	19	48	87	+			+
(1♀)	Width of scutum of female	28	03	32	50	92				+
(8♀)	Length of genu of female	–04	12	32	75	93				++
(9♀)	Length of tibia of female	49	–08	32	28	86	+			
(13♀)	Width of basis capituli of female	20	–01	49	44	93			+	+
(14♀)	Length of palp of female	01	–06	83	19	85			+++	
(15♀)	Length of hypostome of female	00	–03	83	17	85			+++	

Factors	I	II	III	IV
I	X	10	60	69
II	10	X	43	18
III	60	43	X	51
IV	69	18	51	X

Explanation: The upper matrix gives the magnitude of the primary pattern coefficients. The communality (h^2) is the percentage of variation due to common factors for each character. At the right is an abstract of the more important primary pattern coefficients. Plus signs represent the following magnitudes of coefficients: 4 signs ≥ 1.00; 3 signs ≥ 0.80; 2 signs ≥ 0.60; 1 sign ≥ 0.40. The lower matrix gives the correlations among the four factors. Decimal points have been omitted except where the value is equal to or greater than one.

The correlation matrix was factor analyzed in the manner previously described and results are given in Table 18. Interestingly there are four factors, three similar to larval factors obtained in factor analysis of the interlocality component matrix (Table 14) and a new factor that influences only adult characters. Factor I resembles factor I from the larval analysis and affects strongly the width of scutum and basis capituli of larvae and less strongly the width of basis capituli of males and females. The length of genu III and tibia III have high loadings on factor II comparable to factor II from the larval study. Factor III is similar to factor III from the larval study, strongly influencing the length of palp and hypostome of larvae and females but not of males. This reflects the greater morphological similarity between larvae and females than between larvae and males. Factor IV is an adult factor and has no homologue in the previous primary pattern matrix of characters of larvae. The length of genu III and tibia III of the male have the highest factor coefficients on factor IV. Other appreciable scores are found for width of scutum of the male and length of genu III of the female.

d. Section Summary

The hierarchic structure of the data permitted calculation of intralocality product-moment correlation matrices and interlocality product-moment and component correlation matrices. Cluster analysis and principal axis factor analysis with rotation to simple structure summarized patterns of covariation in the three matrices. The interlocality correlations were higher than intralocality coefficients but showed related patterns of covariation. At both levels, high correlations appeared to be morphologically localized or at least of a regional type in that anatomically adjacent or functionally related characters were influenced by the same factors.

The three types of correlation matrices were calculated for larvae, males and females, allowing for comparisons of patterns of correlation within as well as among life history stages.

The number of larval characters employed in subsequent studies (Thomas, 1967) was reduced from 16 to six by selecting the two characters most highly loaded on each of the three interlocality factors. The trends of variation exhibited by these six characters adequately represent the patterns of all 16 characters.

The joint interlocality covariation of larvae, males and females for 33 localities reflected the covariation patterns illustrated by separate analyses for each life history stage. The three larval factors re-emerged with additional loadings of homologous adult characters on two of the factors. The larval appendage factor did not affect adult characters. In addition, a fourth adult factor appeared in this analysis and is best represented by segments of the third leg.

Possible explanations for covariation patterns are discussed below.

DISCUSSION

a. Sources of Variation

The interlocality variation of larval characters was in excess of variation of characters of larvae from different host individuals within a locality. The sources of interlocality variation are likely to be genetic as well as environmental. Design of the analysis did not permit separation of genetic from environmental effects but limited evidence from comparison of laboratory reared and wild-caught adults is suggestive of a genetic basis for some of the observed variation.

The variation of larval characters among host individuals within a locality may be due to inexact locality information, i.e., tick samples having same locality designation may actually be samples from many local populations within a sampling area of variable dimension. However, this variation probably illustrates genetic and environmental differences resulting from restricted gene flow in tick populations because of interaction of behavioral patterns of tick and host.

The tendency of lagomorph hosts to return to resting forms during the daylight hours coupled with the circadian rhythm of drop-off of engorged ticks from the host (Hooker, 1908; George, 1964a, 1964b) may result in the localization of tick populations in the area of a host burrow or form, limiting the spread of tick populations to resident host individuals.

Engorged females dropping onto the host lay their eggs in clusters, increasing the probability that emerging larvae will remain together and eventually encounter the same host. If large numbers of sibs remain together throughout their development, there is increased probability of mating with one another when the adult stage is reached, producing inbred populations and eventually relatively high character variance of larval ticks among host individuals by comparison with variance within such individuals.

Cultivation of land for agricultural purposes produces discontinuities in habitat suitable for the host species resulting in additional isolation of local host populations and their parasites. Random fixation of various genes in these partially isolated tick populations may lead to morphological differences.

There appears to be no morphological differentiation of *H. leporispalustris* into races by adaptation to different host species. The added variability of larval tick populations from migratory birds as compared to resident host species and lack of variation of larvae from different resident host species would indicate that larval morphology is not affected by differences among hosts but that migratory birds are effective in transporting ticks from one geographic area to another.

Different individuals of migratory bird hosts and their tick populations may have had separate geographical origins. Considering the length of time that the engorging tick spends on the host and rates of migration of birds (Lincoln, 1950), dispersal of ticks can occur over considerable distances. *Haemaphysalis leporispalustris* cannot complete its life cycle on a bird but must feed on a lagomorph to produce eggs. Undoubtedly some of the ticks which drop from birds do successfully make the transfer to a lagomorph host, but those larvae that feed on rabbits and aggregate in resting forms through the normal circadian drop-off rhythm would be at a decided advantage in securing a host. Moreover the genotype of migrant ticks may be poorly adjusted to conditions of new surroundings, further reducing their chances of survival. Thus the effective rate of gene flow through migratory hosts may not be appreciable. Because the majority of bird species would be migrating south in the fall when larvae and nymphs are most abundant (Stannard and Pietsch, 1958; Eddy, 1943; Green, *et al.*, 1943; Joyce and Eddy, 1943; Portman, 1944), such gene flow as exist may be unidirectional.

A comparison of variability of homologous characters of larvae, males and females showed adults more variable within and among localities in all characters. Several explanations of why variation of adult ticks *within* a locality should be greater are possible. A sample of adult ticks is more likely to contain genetically unrelated specimens than a sample of larvae, because of high mortality of the immature stages, scattering of sibships by drop-off at different times and places and consequent reattachment to different hosts in later stages. Nutritional factors may contribute to variability of adult ticks since each adult has fed twice before reaching maturity. Ticks feeding on an abnormal host may not reach complete engorgement and, even on the usual host, various factors may interrupt or terminate feeding prematurely. The resulting deficiency in nutrition may affect morphology of subsequent stages. Adult ticks are also older than larvae and have experienced more diverse climatic conditions which may have had an influence on their morphology.

It is more difficult to develop hypotheses to explain the far greater differentiation *among* localities of adult ticks as indicated by the very high coefficients of variation (7.6 to 9.3). Greater selection pressure by environmental factors on adult stages than on larvae may be one explanation. Differences in adults may be the result of allometric growth in various populations and these differences may not be developed in the larval stage resulting in proportionately lower variability among localities of these forms. Localized nongenetic responses to climate, nutritional factors, etc., may also be involved.

The low variation in the hypostome in the three morphological stages may reflect strong selective pressures for uniformity in this character. It is important in attachment of the tick to the host and deviations from the optimal dimension may be disadvantageous.

b. Covariation

While cluster analysis outlines patterns of covariation in the correlation matrices, factor analysis describes the relationships within and among groups of highly correlated variables more precisely.

The intralocality correlations from the 64-locality study of larvae were much lower than interlocality coefficients from the same study. The two matrices showed related but distinct patterns of covariation.

Correlated characters can result from linkage between genes or gene complexes, pleiotropic actions of the same gene complex or nonhereditary common responses to the same environmental factor. Changes in genetic constitution of tick populations and in selective pressures occurring over the range of the species can produce considerable variation in patterns of correlation among localities resulting in lower average correlations within localities and differences between intra- and interlocality covariation. Geographic variation of character correlations has been demonstrated by Clark (1941) and Sokal (1962) in *Peromyscus* and *Pemphigus populi-transversus,* respectively.

The patterns of covariation in intra- and interlocality correlation matrices from the 64-locality study of larvae appeared to be morphologically localized or at least of a regional type in that anatomically adjacent or functionally related characters were influenced by the same factor. The data can be examined for several so-called rules regarding correlation of body parts. Pearson's rule that adjacent organs are more highly correlated than those farther apart applies to leg segment characters in both intralocality and interlocality correlation matrices. Alpatov and Boschko-Stepanenko (1928) stated that correlation is higher between proximal parts of an organ than in distal ones based on studies of correlations of antennae of a hemipteran, *Pyrrhocoris apterus*, and of phalangeal bones of birds. However, in these data correlations of adjacent distal segments of the legs are higher than those observed between proximal segments. During locomotion of ticks most bending of the leg occurs between distal segments so that selection should influence the relative length of these segments more than the less movable basal segments resulting in higher correlations of the former.

Comparison of factor analyses of intra- and interlocality correlation matrices of larvae, males and females showed more similarities between larvae and females than between larvae and males or males and females. Greater similarity between homologous characters of larvae and females can be partially explained as the result of more common functions, and hence related selective pressures, than in characters of males. The scutum of larvae and females is shield-shaped allowing for expansion of the dorsum to permit engorgement. In addition, the palps, hypostome and chelicerae are used in attachment and feeding on the host. The scutum of the male covers the entire dorsal surface and prevents expansion of the integument during feeding. As a result males feed very little or not at all. The chelicerae and hypostome of the male are used in sperm transfer and may have secondary modifications for this purpose. The legs of the male may be used more in locomotion than in larvae and females, as the male actively moves about the host seeking a mate. The legs may also be modified for clasping the females during copulation.

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A Numerical Taxonomic Study of Certain Taxa of *Haplopappus*, section *Blepharodon*[1,2]

SERAFIN RAMON[3]
Botany Department

ABSTRACT

Nine different perennial, diploid taxa ($n = 4$), of *Haplopappus* section *Blepharodon* from distant geographic localities were used to determine the relationships and effectiveness of numerical taxonomy in classification. The study compares numerical taxonomic analyses with hybridization and cytological observations of the nine taxa involved to determine the taxonomic relationships among them. Taxa used were *Haplopappus arenarius* Benth, subsp. *arenarius, H. arenarius* supsp. *incisifolius* (Johnston) Jackson (in ed.) *H. texensis* Jackson, *H. gooddingii* (A. Nels.) Munz and Johnston, and five different subspecific taxa of *H. spinulosus.*

INTRODUCTION

The *Blepharodon* section of the genus *Haplopappus* contains two annual and a number of perennial taxa according to the latest revisions of Hall's (1928) treatment. Changes, additions and exclusions, have been made in the section by Jackson (1962a, b), Munz and Keck (1959), and others. Although some tetraploid taxa have been found, the majority of the perennial taxa included in the section have haploid chromosome numbers of $n = 4$. The basic chromosome number of $X = 4$ was established for the section by Jackson

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[2] This work was based on a dissertation submitted to the Graduate School of the University of Kansas in partial fulfillment of the requirements for the degree Doctor of Philosophy.

Sincere appreciation is expressed to Dr. Raymond C. Jackson for his assistance during the course of the research and preparation of the manuscript.

[3] Present address: Department of Biology, Panhandle State College, Goodwell, Oklahoma 73939.

(1962a). Nine different diploid taxa ($n = 4$) were chosen from distant geographic localities to determine their relationships and the effectiveness of certain taxonomic approaches as a basis of classification.

The number of studies in which plants were used in numerical taxonomic (NT) treatments is limited. Among the first was that of Rogers and Tanimoto (1960) using herbarium specimens of *Manihot esculenta*. Others that utilized this approach were Soria and Heiser (1961) and Heiser *et al.* (1965) with the genus *Solanum*, Morishima and Oka (1961) with *Oryza*, and Katz and Torres (1965) with *Zinnia*. Wirth, Estabrook, and Rogers (1966) also applied NT analyses in their work with orchids. Crovello (1966) applied this type of study to the section *Sitchenses* of the genus *Salix*, and a complete taximetric study of the section *Apoucouita* of the genus *Cassia* has been published recently by Irwin and Rogers (1967). This latter monographic study was applied to herbarium specimens collected from South America. Gilmartin (1967) provides a comprehensive list of references on NT studies in botany.

The techniques used in applying NT or taximetrics (Rogers, 1963) to organismal classification were developed and promoted principally by Michener and Sokal (1957), Sokal (1961, 1962, 1963), Sokal and Sneath (1963), and Rohlf and Sokal (1965). Since its development the majority of the studies in which numerical analyses were used involved organisms other than plants (Rohlf, 1963, 1965; Michener and Sokal, 1966; and Sokal and Michener, 1967). These reports describe efforts to classify taxa and to compare the results of numerical taxonomy generated by using several different methods.

The papers mentioned above in which plants were used also were of a comparative nature. Heiser *et al.* (1965) worked with members of the genus *Solanum* which had been intensively studied and classified by conventional means and the results were compared to those produced by numerical analyses. Studies on the cespitose zinnias by Katz and Torres (1965) compared the results of three different numerical techniques to those that Torres had previously obtained from cytogenetic and chromatographic studies. The works of Sokal and Michener (1967) on the bee genus *Hoplitis* and those of Rohlf (1963) on *Aedes* mosquitoes were primarily concerned with the effects of different numerical techniques on their classification.

Rohlf and Sokal (1965) discussed the merits of using coefficients of correlation and distance as the similarity matrix in generating phenograms and concluded that under certain conditions (standardization of data) these two coefficients provided approximately equal results. As these two types of coefficients were used in the present study, particular interest will be placed on the phenograms produced by each coefficient. Rohlf (1963) found that a more satisfactory treatment of *Aedes* could be obtained if the distance coefficients were used. However, Moss (1966) in his work with the martin mite, *Dermanyssus prognephilus,* concluded that a combination of phenograms using both coefficients of correlation and distance provided the better results.

The present study utilizes both of these types of coefficients to obtain phenograms and a three-space projection for each of the four data matrices developed for use. Two numerical analyses were used here to obtain an estimate of the phenetic patterns of variation among the operational taxonomic units (OTU's) in the context of the characters used. Other workers have used numerical taxonomic classifications to compare with those derived by other means.

MATERIALS AND METHODS

Parental and Hybrid Taxa. Nine different taxa of the genus *Haplopappus* that had previously been collected and maintained in a greenhouse were used in the present study. These taxa were crossed reciprocally to obtain F_1 hybrids using the technique described for the genus by Jackson (1962a).

The lenspaper covered heads were allowed to remain on the plants after pollination for 3 to 4 weeks and then collected. Seeds were stored in a refrigerator at 15 C° for 6 to 10 weeks. After this period they were germinated in water, allowed to grow for 3 to 5 days then planted in "jiffy-pots" which were later placed in 6 inch clay pots in the greenhouse.

The taxa used in the study were *Haplopappus arenarius* Benth. subsp. *arenarius, H. arenarius* subsp. *incisifolius* (Johnston) Jackson (in ed.), *H. texensis* Jackson, *H. gooddingii* (A. Nels.) M. & J., and five different collections of *H. spinulosus* Pursh. The latter taxon contains a number of subspecies as presently treated by the most extensive work on the genus (Hall,

TABLE 1. Collection data for plants used in the present study.

Taxon	Date	Locality
Haplopappus arenarius subsp. *arenarius*	1961	Cape San Lucas, Baja, California
Haplopappus arenarius subsp. *incisifolius*	3-24-1962	South San Lorenzo Island, Gulf of California
Haplopappus texensis	8-25-1957	7 mi. south of Falfurrias, Brooks Co., Texas
Haplopappus gooddingii	5-09-1959	Hoover Dam, Clark Co., Arizona
Haplopappus spinulosus (2611)	6-12-1958	Tejaris Canyon, Bernalillo Co., New Mexico
Haplopappus spinulosus (2737)	9-06-1959	West of Monoclova, Coahuila, Mexico
Haplopappus spinulosus (2769)	8-08-1959	3 mi. west of Saltillo, Coahuila, Mexico
Haplopappus spinulosus (2998)	9-08-1959	12 mi. north of Ransom, Trego Co., Kansas
Haplopappus spinulosus (3009)	6-10-1960	11.9 mi. south of Vernon, Wilbarger Co., Texas

1928), but in the present study they will be referred to by the collection numbers of R. C. Jackson. Additional information on these collections are given in Table 1.

Five plants of each parent and hybrid were grown for use in the study; in some instances, poor germination and other factors resulted in fewer than five plants. A number of crosses failed to yield viable seeds, and as a result some hybrids were not available for these analyses. Code numbers of parent taxa and hybrids are listed in Table 2.

Pollen Fertility. Pollen fertility for the parent taxa and the F_1 hybrids was determined by pollen stainability, using 5 percent cotton blue in lactophenol. Only those grains with uniformly stained cytoplasm and having a round or oval shape were considered viable. A total of 500 pollen grains per plant were counted in determining the fertility percentages. Whenever possible only terminal heads were used in taking pollen samples.

Cytological Techniques. Immature heads were collected between 11:00 a.m. and 12:30 p.m. and fixed in Carnoy's fluid for at least 24 hours. Individual flowers of appropriate size were taken from the heads and prepared

TABLE 2. List of code numbers for OTU's of parental taxa and hybrids used in present study.

1. *arenarius* × *incisifolius*
2. " × *texensis*
3. " × 2611
5. " × 2737
6. " × 2769
7. " × 2998
8. " × 3009
9. *incisifolius* × *arenarius*
11. " × 2611
13. " × 2737
14. " × 2769
15. " × 2998
16. " × 3009
18. *texensis* × *incisifolius*
19. " × 2611
20. " × *gooddingii*
21. " × 2737
22. " × 2769
23. " × 2998
24. " × 3009
25. 2611 × *arenarius*
26. " × *incisifolius*
27. " × *texensis*
29. " × 2737
30. " × 2769
34. *gooddingii* × *incisifolius*
35. " × *texensis*
36. " × 2611
37. " × 2737
38. " × 2769
39. " × 2998
40. " × 3009
41. 2737 × *arenarius*
42. " × *incisifolius*
43. " × *texensis*
45. " × *gooddingii*
46. " × 2769
49. 2769 × *arenarius*
50. " × *incisifolius*
51. " × *texensis*
52. " × 2611
53. " × *gooddingii*
54. " × 2737
56. " × 3009
57. 2998 × *arenarius*
58. " × *incisifolius*
59. " × *texensis*
61. " × *gooddingii*
65. 3009 × *arenarius*
66. " × *incisifolius*
67. " × *texensis*
68. " × 2611
69. " × *gooddingii*
70. " × 2737
71. " × 2769
75. *Haplopappus arenarius* subsp. *aren.*
76. *Haplopappus arenarius* subsp. *incis.*
77. *Haplopappus texensis*
78. *Haplopappus spinulosus* (2611)
79. *Haplopappus gooddingii*
80. *Haplopappus spinulosus* (2737)
81. *Haplopappus spinulosus* (2769)
82. *Haplopappus spinulosus* (2998)
83. *Haplopappus spinulosus* (3009)

for examination by the established squash technique. Propiocarmine stain was used with a few drops of saturated ferric acetate added as a mordant. Chiasma frequency counts of developing microsporocytes were made from meiotic chromosomes at diakinesis at 970×. In most instances 50 cells were counted for each plant in which chiasmata frequencies were taken.

Morphological Measurements and Character States. All measurements of character states used in the numerical taxonomic analyses were taken from fresh material. Only terminal heads in which at least one-half of the disc flowers had opened were used in securing ray and disc flower and phyllary measurements. The leaves, five from each plant, were taken from the midstems of prominent lateral or main axis stems. In some instances the leaves were stored in petri dishes containing moist filter paper until measurements could be made. Length and width measurements were taken in millimeters with a vernier scale under a binocular dissecting microscope. A filar micrometer mounted on a compound microscope was used to obtain pollen grain diameters. Twenty-nine characters were originally obtained for each plant. One of these characters was found to be invariant and was omitted in the numerical analyses. Of the 28 characters ultimately used, 17 were measured characters and 11 were coded. The characters that were used are the following: disc flower number, disc flower length, disc flower achene length, disc flower achene pubescence, disc flower pappus length, disc flower stigma lobe length, ray flower number, ray flower ligule length, ray flower ligule width, ray flower achene length, ray flower pappus length, head disc diameter, number of phyllary rows (series), phyllary length, phyllary width, phyllary tip curvature, leaf length, leaf width, leaf type, leaves with more than one dissection, upper leaf surface pubescence, lower leaf surface pubescence, stem pubescence, stem vesture, presence or absence of basal rosette, pollen grain diameter, ray flower color, presence or absence of alveolate bracts.

Leaf pubescence was scored on the basis of five alternative categories: (1) entirely glandular, (2) entirely filiform, (3) approximately 50-50 glandular-filiform, (4) mostly glandular, and (5) mostly filiform. Determinations were made by counting the type and number of hairs within a square of an ocular grid. Stem pubescence was similarly recorded, but the portion of the stem used was 3 to 5 inches below the terminal head.

Pubescence on the achenes was scored in three character states: (1) light pubescence, where the entire achene surface could be seen, (2) medium pubescence, where only part of the achene surface could be seen, (3) extremely pubescent, where only little or none of the achene surface was visible.

Stem vesture was divided into two categories: villous or pilose as defined by Lawrence (1964). Two characters, basal rosette and alveolate bracts, were recorded as to whether they were present or absent. Phyllary measurements were taken from the innermost series as they appeared underneath a cover slip on a slide and under the magnification of a dissecting microscope.

Floral color determinations were made with the use of a Nickerson color fan (Munsell Color Co.). Five character states were used: (1) 2.5Y 8/12, vivid yellow, (2) 5Y 8/12, vivid yellow, (3) 7.5Y 8/12, vivid greenish yellow, (4) 7.5Y 9/8, brilliant greenish yellow, and (5) 10Y 9/9, vivid greenish yellow.

The nine parental taxa exhibited six basic leaf types (Fig. 1), and these were used as the code states in scoring the type of leaves for the hybrids.

Numerical Taxonomic Studies. Measurements for 28 characters were made on 255 greenhouse grown plants. Four different data matrices were derived from these measurements. One represented each of the 255 individual plants as an OTU, while a second used only the means of the parent and hybrid plants, resulting in 64 OTU's. The two remaining data matrices were the same as the above two except that the hybrid plants were omitted.

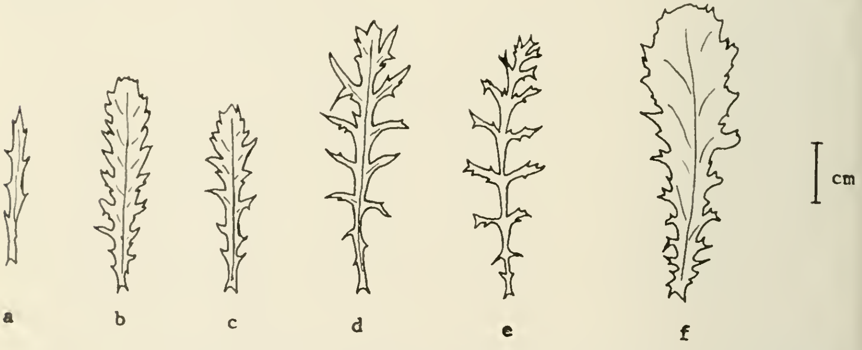


FIG. 1. The six basic leaf types exhibited in the nine parent taxa, Fig. 1a, *Haplopappus gooddingii*. Fig. 1b, *H. texensis*. Fig. 1c, 2737 and 2769. Fig. 1d, *H. arenarius* subsp. *incisifolius*. Fig. 1e, 2611, 2998, and 3009. Fig. 1f, *H. arenarius* subsp. *arenarius*.

Two numerical analyses were made with each of the four data matrices. The first analysis produced a distance phenogram and a correlation phenogram. To obtain these, the data matrices were first standardized by characters (Rohlf and Sokal, 1965). This procedure gives each character approximately equal weight. From the standardized character values two matrices were computed. One was a distance matrix using a modification of Sokal's coefficient of distance,

$$\text{dist.}_{jk} = \left[\sum_{i=1}^{n} \frac{(X_{ij} - X_{ik})^2}{n} \right]^{\frac{1}{2}},$$

Sokal and Sneath (1963). The second was a character correlation matrix computed in the manner of Sokal and Sneath (1963). The distance and correlation matrices were then used to cluster the different OTU's employing the unweighted pair-group method using arithmetic averages. Phenograms were then produced as a result of the various clustering cycles. A cophenetic correlation coefficient was then calculated by comparing the cophenetic values generated in making the phenograms with the original distance and correlation matrices. This correlation indicates the amount of distortion produced in the construction of the phenograms. The closer the correlation value is to 1.0 the less the distortion (Sokal and Sneath, 1963).

In the second analysis, three-dimensional projection values were calculated by computing a character correlation matrix from the data matrix. A principle component analysis was made on the character correlation matrix in which the first three components were extracted using the centroid method. The correlations of each character with these three components was then used in obtaining the projected values of the OTU's in the three-space. The distances between the OTU's in the principle component three-dimensional space was calculated from the projected values. To check the degree of agreement between the projected distances calculated using the three centroid axes and the distance matrix of 28 characters, the correlation between the two distances matrices was calculated. A correlation value approaching 1.0 would indicate how accurately the three-space projections reflect the relationships in the 28 character space.

The numerical taxonomic procedures used were developed by F. J. Rohlf, John Kishpaugh, and Ron Bartcher of The University of Kansas. All computations were made at The University of Kansas Computation Center using a GE 625 Digital Computer. Copies of the different data matrices employed in the study will be on file at the Department of Botany of The University of Kansas and will be available to interested persons.

TAXONOMY OF THE PARENTAL TAXA

The taxonomy of the nine taxa used in the study is not clear. The most recent treatment of the entire genus of *Haplopappus* is that of H. M. Hall (1928). Since this work, other investigators have proposed several changes in his classification. Hall placed all taxa used in the present study under the section *Blepharodon*. He lists *H. arenarius* as a distinct species which occurs only in the Cape Region of Baja California. *H. gooddingii,* and *H. arenarius* subsp. *incisifolius* are treated as subspecies of *H. spinulosus*. Hall refers to the latter as "a polymorphous species of wide distribution, especially notable for the range of variation in foliage." Since Hall's treatment, subspecies *gooddingii* has been designated as a species by Munz and Johnston (1959). Johnston (1924) had previously designated *incisifolius* as a variety of *H. arenarius*. Jackson (unpublished) considers this taxon as a subspecies of

H. arenarius. H. gooddingii has a listed distribution of southern Nevada, northern Arizona, and southeastern California while *H. arenarius* subsp. *incisifolius* has a more restricted distribution on certain islands in the Gulf of California.

Haplopappus texensis, a recently described species proposed by Jackson (1962), is one of the most phenotypically distinct taxa used in the present study. It is at this time known only from the type locality in Brooks County, Texas, some 7 miles south of Falfurrias.

The other five taxa utilized, designated by the collection numbers of Jackson, are 2611, 2737, 2769, 2998, and 3009. These would, in most instances, be classified as belonging to the *H. spinulosus* complex. They are similar in some characters but quite dissimilar in others. No doubt all of the taxa listed above could be assigned to one or more of the subspecies that Hall had designated for *H. spinulosus*, but no attempt to do so was made here because Hall's key groups many morphologically and cytologically diverse plants in the same subspecies.

CYTOLOGICAL OBSERVATIONS OF PARENTAL TAXA AND HYBRIDS

All nine taxa of *Haplopappus* have a diploid chromosome number of eight. Raven *et al.* (1960) reported the haploid number for *H. gooddingii* as $n = 4$. Jackson (1957) published the haploid number of several subspecies of *H. spinulosus* as $n = 4$. Included among these were some of the *H. spinulosus* taxa considered in the present study. The haploid chromosome number for *H. texensis* was also reported by Jackson (1962a). The $n = 4$ counts for *H. arenarius* subsp. *arenarius* and subsp. *incisifolius* and the two Mexican taxa, 2737 and 2769, have been established by Jackson (unpublished).

The hybrids resulting from the reciprocal crosses had a haploid chromosome number of $n = 4$. There was only a single exception; one of the plants examined in the 2611 × *H. arenarius* subsp. *incisifolius* cross proved to be a tetraploid with $n = 8$. A study of this plant revealed that eight bivalents were present in most cells examined. In a few cells, multivalents seemed to have occurred. Some of the pollen grains of the plant were tetracolpate, a characteristic seen in naturally occurring tetraploid species of *Haplopappus*, whereas the normal condition for the genus is tricolpate grains. Micropollen grains were also found.

The diploid chromosome complement of the nine taxa from all observations are identical. Each is composed of two meta- to submetacentric chromosomes which are shorter than the three pairs of acrocentric ones. One pair of the acrocentric chromosomes had a secondary constriction and a satellite present. The idiogram (Fig. 2) shows a representative karyotype which all the parental taxa possess.

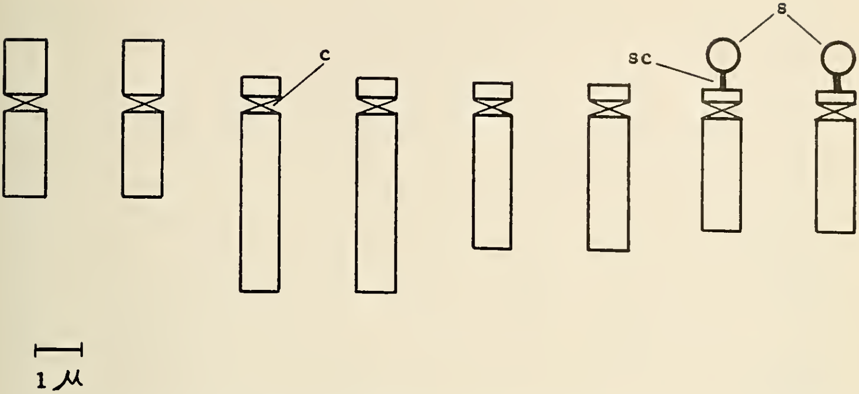


FIG. 2. Chromosome idiogram representative of the karyotype found in the nine parent taxa. s=satellite, sc=secondary constriction, c=centromere.

Meiotic configurations in all parental taxa and hybrids were normal with all possessing four bivalents at diakinesis and early metaphase I. The longer of the acrocentric chromosomes, in some instances, had delayed disjunction at anaphase I. However, no fragments were ever found, and after close examination of these configurations it was decided that they did not represent a dicentric bridge. The same condition was also noted in a few cells at anaphase II. Critical examination of microsporocytes with such configurations at pachytene stages failed to reveal any unpaired regions or aberrations.

All parental and hybrid taxa had at least one chiasma per bivalent. Ring bivalents which were taken to have one chiasma on either end of the chromosome pair were not consistently found in any of the parental taxa. This can be seen in that the average minimum chiasma frequencies were all below five per cell (Table 3). The chiasma frequency values per cell were, however, relatively close to five. The lowest frequency values were found in *H. texensis* (4.69) and the New Mexico taxon 2611 (4.65).

Several of the F_1's did have minimal chiasma frequencies that were above five per cell. These were 2769 × *arenarius,* 2611 × *texensis, incisifolius* × 2737, *incisifolius* × 2769, *texensis* × 2737, *texensis* × 2769, *gooddingii* × 2769, 2737 × 2769, *arenarius* × 2998, *incisifolius* × 2998, *texensis* × 2998, *incisifolius* × 3009, *texensis* × 3009, and 2768 × 3009 (Table 3). It should be noted that most of the hybrids mentioned above have as one of the parents a taxon that is considered part of the *H. spinulosus* complex. In the majority of these crosses, the *H. spinulosus* complex parent was the staminate plant while the pistillate parents are concentrated in the *arenarius, incisifolius,* and *texensis* taxa. This observation tends toward the assumption that the chromosomes of these taxa are homologous over most of their lengths.

TABLE 3. Mean chiasma frequencies in parental taxa and hybrids. When reading from left to right and from top to bottom the first taxon read will be the pistillate parent.

OTU	(75) aren	(76) incis	(77) tex	(78) 2611	(79) good	(80) 2737	(81) 2769	(82) 2998	(83) 3009
(75) ssp. arenarius	*4.91*	4.71	5.01	4.95	-	4.65	4.64	5.41	4.75
(76) ssp. incisifolius	4.53	*4.80*	——	4.72	——	5.15	5.01	5.15	5.26
(77) *H. texensis*	......	4.73	*4.69*	4.85	4.86	5.19	5.06	5.19	5.13
(78) 2611	4.79	4.80	5.07	*4.65*	......	4.78	4.66	......	......
(79) H. gooddingii	......	4.85	4.88	4.73	*4.82*	4.81	5.23	4.63	4.89
(80) 2737	4.85	4.61	4.95	......	4.49	*4.93*	5.29		......
(81) 2769	5.06	4.89	4.95	4.87	4.63	4.83	*4.87*	......	5.08
(82) 2998	4.74	4.76	4.69	——	4.67	......	......	*4.84*	......
(83) 3009	4.94	4.78	5.06	4.62	4.72	4.87	4.69	......	*4.78*

In no instance did any of the hybrid combinations exhibit a chiasma frequency value that was extremely different from either parent. The range of average minimum chiasma frequency per cell was 4.49 for hybrids between 2737 and *H. gooddingii* to 5.19 for *H. texensis* × 2998 (Table 3). The fact that no great disparities in chiasma frequencies occur tend to point to the homology of the different genomes in the various taxa. Sufficient pairing must have occurred to allow crossing over to take place as chiasma are cytological evidence for this occurrence (Swanson, 1957).

Pollen fertility, as determined by stainability, for the hybrids listed above was extremely variable (Table 4). If these taxa show a close genetic relationship, one would expect fairly high pollen fertility in the F_1's unless cryptic structural hybridity was involved (Stebbins, 1950). The average pollen fertility for the five or less plants considered in some of the above hybrid crosses was quite low (Table 4). Eleven hybrids had average fertility percentages that were less than 60 percent, while the highest fertility exhibited by any of these hybrids was just above 75.0 percent. The highest pollen fertility for any one hybrid plant (Table 4) is somewhat higher than the average. However, even if these values are considered, 13 hybrids had a fertility below 70.0 percent and 24 were below 80.0 percent.

The lowest average pollen fertility percentages were found in the following hybrids: 3009 × *H. gooddingii* (37.0), 2769 × 3009 (39.8), 3009 × *incisifolius* (42.3), 3009 × *arenarius* (44.7), and *H. gooddingii* × 3009 (45.9). It should be noted that all hybrids given above have 3009 as one of the parents. The

TABLE 4. Pollen fertility of parental taxa and hybrids, means and range.

OTU	(75) aren	(76) incis	(77) tex	(78) 2611	(79) good	(80) 2737	(81) 2769	(82) 2998	(83) 3009
(75)	*97.0*	82.2	61.2	73.0	......	57.4	67.9	53.2	50.7
aren		72.2-97.0	49.3-67.6	64.2-80.4		46.4-61.6	53.0-79.2	49.8-75.0	35.0-67.4
(76)	72.6	*97.8*	......	60.8	......	74.3	69.0	72.4	58.7
incis	65.8-84.0			53.6-70.4		64.6-84.0	61.4-72.8	63.8-81.3	52.7-69.0
(77)	......	61.9	*99.1*	85.9	65.8	51.0	73.3	80.4	78.0
tex		47.0-70.2		81.2-94.2	55.8-75.8	49.0-64.0	64.4-79.8	73.0-87.6	64.6-91.2
(78)	70.7	60.9	63.7	*98.4*	......	93.0	96.0	......	......
2611	65.4-76.8	49.3-83.8	36.0-95.6			89.6-96.3	92.6-98.0		
(79)	......	61.0	61.5	74.3	*98.0*	89.1	70.5	70.2	45.9
good		57.6-64.3	61.5-......	52.0-84.6		83.3-95.5	60.3-86.7	66.2-81.3	20.2-60.8
(80)	69.0	68.3	80.5	......	90.0	*99.0*	98.5	......	......
2737	59.5-85.8	62.0-87.0	75.0-84.4		86.2-93.8		98.0-99.0		
(81)	62.7	75.1	78.0	97.7	88.3	97.4	*97.4*	......	39.8
2769	52.2-65.6	63.0-87.3	56.2-93.2	97.6-79.7	85.3-92.8	97.2-97.6			11.3-75.1
(82)	61.5	57.6	88.0	......	70.1	......	......	*97.9*	......
2998	52.2-72.2	49.4-63.8	72.6-96.4		64.6-77.4				
(83)	44.7	42.3	93.7	99.2	37.0	66.3	73.1	......	*93.9*
3009	33.2-59.4	31.0-61.0	87.8-95.8	99.2-......	22.7-62.2	29.4-89.6	56.0-92.5		

range of pollen fertility for each hybrid as well as the averages are given in Table 4.

Several of the hybrid combinations were noticed to have produced micropollen grains. In certain plants of a few hybrids, these pollen grains were extremely numerous. The greatest number was found in one of the plants of the *H. gooddingii* × 3009 crosses where the majority of the nonstained grains were of this type. Pollen fertility for this particular plant was quite low (27.8). Similar situations were found in one plant of 3009 × *H. gooddingii* (28.4) and 3009 × 2769 (56.0) crosses. These plants, and others having low pollen fertility percentages, were examined carefully at pachytene stages for unpaired chromosome regions, but none were found. In several crosses where micropollen grains were noted, fertility was quite high (Table 4). Some ran as high as 90 percent or higher while a number of hybrids were between 75 and 90 percent.

NUMERICAL ANALYSES

Two different numerical analyses were generated on the parental and hybrid taxa in the present study. These analyses produced two different phenograms and a set of values for the placement of the OTU's in a three-space for each of the four data decks used. In the following discussion, the phenograms generated from the coefficients of correlation will be referred to as the correlation phenograms while those computed from the coefficients of distance will be called the distance phenograms. The results of the 64 OTU (parent and hybrid means), 26 OTU (individual parent plants), 9 OTU (parent means), and the 255 OTU (each individual plant) distance and correlation phenograms will be given and compared. Particular emphasis will be placed on the position of the parental OTU's and the clustering of the hybrids.

The correlation and distance (cophenetic) values shown on each phenogram indicate the relationship between any two OTU's (Sokal and Sneath, 1963). On the correlation phenograms the higher the value the closer the relationship, while the lower values on the distance phenograms indicate the closer affinity.

For each of the phenograms a cophenetic correlation coefficient was computed. This correlation was calculated by comparing cophenetic values generated in making the phenogram with the original data matrix and indicates the amount of distortion produced in constructing the phenogram. The correlation (r) values for the phenograms are shown in Table 5.

Table 5. The cophenetic correlation coefficients of the analyses.

Analysis	Correlation Phenogram	Distance Phenogram
9 OTU	0.734	0.815
26 OTU	0.877	0.847
64 OTU	0.616	0.715
255 OTU	0.772	0.988

These values should be taken into consideration as one views the phenograms. The correlation values would indicate that the 255 OTU distance phenogram reflects the more accurate relationships between OTU's in light of the 28 characters. In three out of the four sets of phenograms, the distance phenograms showed higher correlation values than the correlation phenograms. Only the 26 OTU correlation phenogram had a higher value than the distance phenogram.

64 OTU Correlation Phenograms

Figure 3 is the correlation phenogram of the 64 OTU matrix. Four major clusters can be easily distinguished. OTU's 75 (subsp. *arenarius*) and 76 (subsp. *incisifolius*) are grouped relatively close to each other. Clustered with them are two hybrids in which both serve as one parent. This small cluster is joined to a small group of hybrids in which OTU's 75 or 76 serves as one of the parents.

All of the *H. spinulosus* complex OTU's are placed within a single major cluster. OTU 80 (2737) and 81 (2769) were grouped close to one another. The degree of their relationship is indicated by the high correlation values expressing their union (Fig. 3). OTU 81 was first clustered with several hybrids where it served as the pistillate parent in four crosses and staminate parent in two hybrids. OTU 80 was initially joined with three hybrids

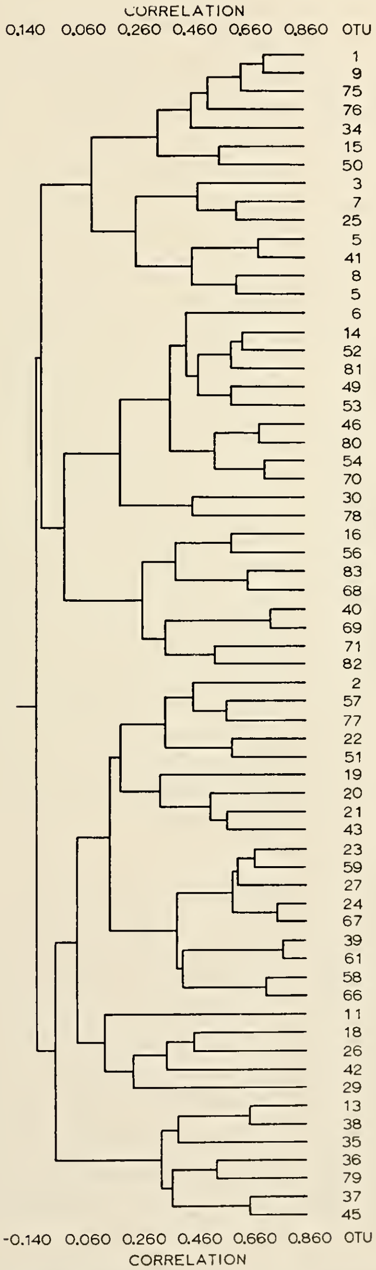


FIG. 3. 64 OTU Correlation Phenogram. The OTU's represent mean values for the nine parent taxa and hybrids. The level values shown on the phenogram are the correlation values at which OTU's cluster together.

where it too served as one parent before the two groups were joined to form a single cluster. OTU 78 (2611) clustered first with OTU 30 (2611 × 2769) then joined a cluster of ten OTU's which contained OTU's 80 and 81. Taxon 3009 (OTU 83) was clustered with OTU 82 (2998) to form a minor cluster with several hybrids in which OTU 83 was either the pistillate or staminate parent. The clustering of OTU 82 within this complex is not surprising as it has several characteristics that are similar with OTU 83 (3009). As to why hybrids in which OTU 82 was a parent were not included in the cluster is not known. One would expect a few hybrids of this type to be included in the grouping. A cluster of eight OTU's (16, 56, 83, 68, 40, 69, 71, and 82) was then joined to the cluster of ten OTU's which contained OTU's 78 (2611), 80 (2737), and 81 (2769). The major cluster formed as a result of this union was then joined to the cluster in which OTU's 75 (subsp. *arenarius*) and 76 (subsp. *incisifolius*) were found. (Table 2 lists the code numbers of the different hybrid crosses as well as the parental taxa.)

The third major cluster of the 64 OTU correlation phenogram has thirty OTU's that were grouped in four minor clusters. In this large constellation are located the two remaining parental taxa, *H. texensis* (OTU 77) and *H. gooddingii* (OTU 79). These two OTU's are not, however, too closely related as can be noted in the correlation value on the phenogram (–0.100). OTU 79 is grouped with five OTU's in which it serves as one of the parents. The high correlation values indicated show that these OTU's are quite close phenetically (Fig. 3). This minor cluster joins three other minor clusters that had previously been grouped. One of the three latter clusters contained OTU 77 *(H. texensis)*. It is grouped with nine other OTU's in which it was either the pistillate or staminate parent.

64 OTU Distance Phenogram

The 64 distance phenogram differs from the correlation phenogram in several respects. Essentially it possesses only two major clusters (Fig. 4). The largest of these clusters contained all parental taxa except for OTU's 75 (subsp. *arenarius*) and 76 (subsp. *incisifolius*). OTU's 75 and 76 are placed with the hybrids in which both are on the parents. The relatively low distance values in the cluster would indicate a fairly close relationship among these OTU's. This is similar to the results in the correlation phenogram. The distance value at which this cluster joins the large cluster would indicate a distant relationship between these OTU's and the other parental or hybrid OTU's.

OTU 77 *(H. texensis)* was grouped with seven hybrids in which it was one of the parents. The closest parental taxon to OTU 77 was OTU 82 (2998). The high distance value at their union indicates, however, that the

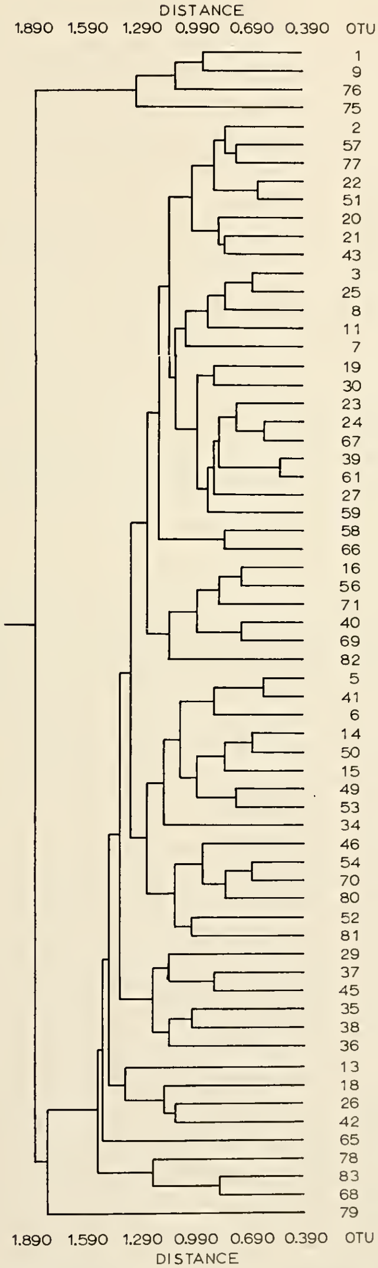


FIG. 4. 64 OTU Distance Phenogram. The OTU's represent mean values of the nine parent taxa and hybrids. The level values shown on the phenogram are the distance values at which OTU's cluster together.

two are only distantly related. OTU 82 was clustered with five hybrid OTU's (16, 56, 71, 40, 69), none of which have OTU 82 as a parent. This same situation was also noted in the 64 OTU correlation phenogram. Those hybrids were OTU 82 (2998) did serve as a parent were placed closer to OTU 77 *(H. texensis)* as seen in Figure 4. OTU's 80 and 81 are shown to be more closely related to each other than to any other parent taxa. They show a closer affinity to OTU's 77 and 82 than to the other parent OTU's. Both OTU 80 and 81 were clustered with several hybrids in which one of the other served as a parent. OTU's 78 (2611) and 83 (3009) are clustered with a single hybrid, 3009 × 2611. The distance value would indicate that these two are fairly closely related. The two show a closer affinity to other *H. spinulosus* complex OTU's and OTU 77 *(H. texensis),* as would be expected, than they do to OTU's 75 (subsp. *arenarius*) and 76 (subsp. *incisifolius*).

The 64 OTU distance phenogram depicts OTU 79 *(H. gooddingii)* as being quite distant from OTU 77 and the OTU's of the *H. spinulosus* complex. It does show a closer relationship to those OTU's than it does to OTU's 75 and 76.

26 OTU PHENOGRAMS

The phenograms generated when the individual plants of the parent taxa were considered as OTU's showed differences when compared with each other and the other phenograms (Figs. 5, 6). Both the correlation and distance phenograms showed that OTU's 75 and 76 are more closely related to each other than to other parent OTU's and separates them distinctly from the other taxa. The differences in the two phenograms was in the clustering of the remaining parental OTU's.

The correlation phenogram shows a small cluster in which OTU 77 *(H. texensis)* is grouped with OTU's 78 (2611), 82 (2998), and 83 (3009). Within the cluster the correlation values indicated that OTU 82 and 83 are more closely related. These two OTU's then show a closer affinity to OTU 78 (2611) than they do to OTU 77. The cluster with OTU's 77, 78, 82, and 83 joins a minor cluster containing OTU's 79 *(H. gooddingii)*, 80 (2737), and 81 (2769). Within the latter cluster OTU's 80 and 81 are shown to be most closely related. The relatively high correlation value (0.220) clearly indicate this relationship (Fig. 5). This major cluster, formed by the union of the two minor clusters mentioned above, is then joined with the cluster containing OTU 75 and 76. This placement would indicate that OTU 77 has a closer relationship to OTU's 78, 82, and 83 than to OTU's 79, 80, and 81. These same relationships can also be seen in the three-space projection (Figs. 8, 9, 10).

The placement of OTU's on the distance phenogram differs from those seen in the 26 OTU correlation phenogram (Fig. 6). This distance pheno-

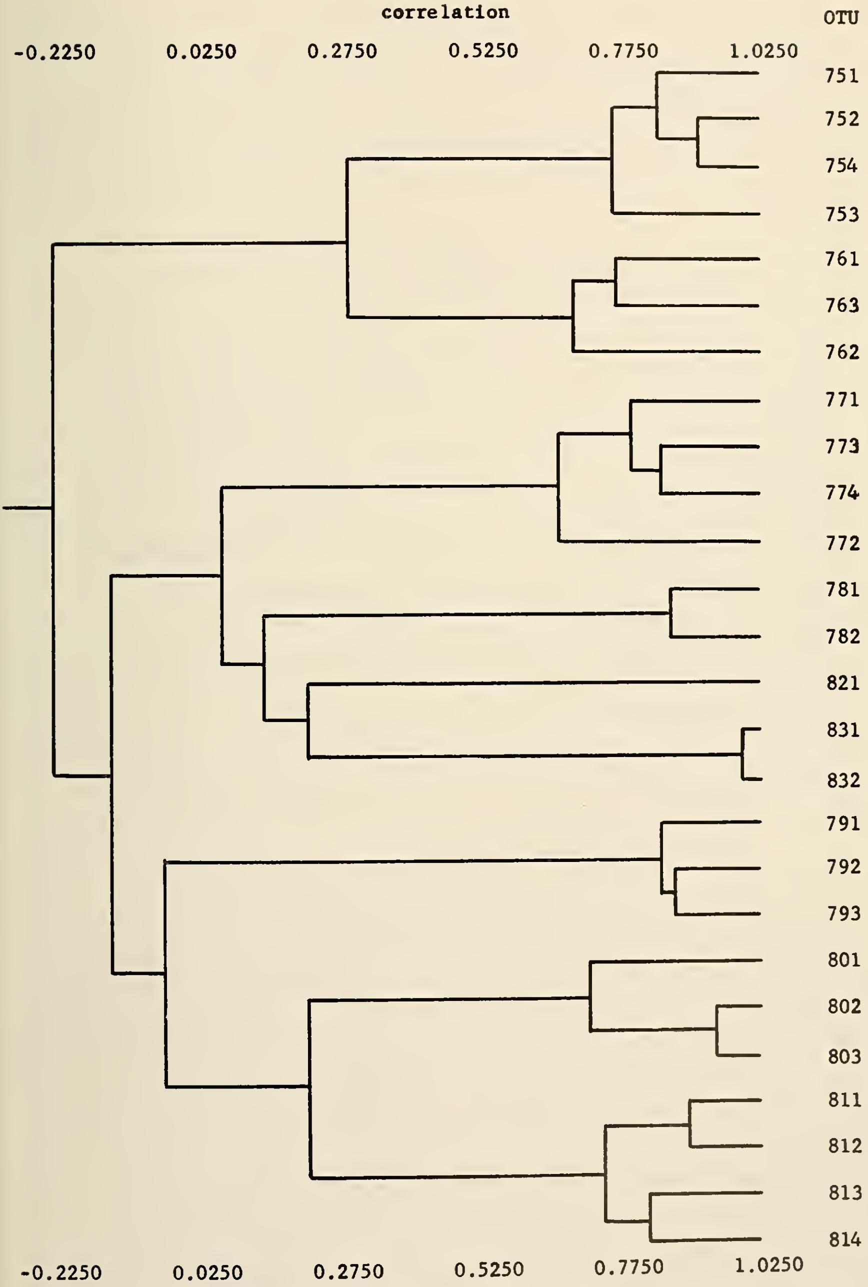


FIG. 5. 26 OTU Correlation Program. Each OTU in the phenogram represents an individual parent plant. The third digit on the OTU number represents the number of the individual parent plants.

FIG. 6. 26 OTU Distance Phenogram. Each OTU in the phenogram represents an individual parent plant. The third digit on the OTU number represents the number of the individual parent plants.

gram shows that OTU's 82 (2998) and 83 (3009) are more closely related to OTU 77. However, it also reveals that OTU's 80 and 81 have a closer affinity to the cluster containing OTU's 77, 82, and 83 than they do to OTU 78 (2611). This is quite different from the relationships expressed in the correlation phenogram. The distance phenogram also indicates that OTU 79 is distinct from OTU's 77, 82, 81, and 78.

9 OTU Phenograms

The two phenograms produced from the mean values of the parent taxa are shown in Figure 7. Both the correlation and distance phenograms show the separation of OTU's 75 (subsp. *arenarius*) and 76 (subsp. *incisifolius*) as did the 26 and 64 OTU phenograms. Differences in the two phenograms are in the placement of OTU's of the *H. spinulosus* complex and OTU 79 *(H. gooddingii)*. OTU 77 *(H. texensis)* is clustered with OTU's 82 (2998) and 83 (3009) in the correlation phenogram. In addition, this phenogram clusters OTU's 80 and 81 to each other then joins them to OTU 78 (2611). This cluster of three OTU's is then joined to OTU 79 *(H. gooddingii)*. This four OTU cluster was then joined with the cluster containing OTU's 77, 82, and 83. These placements would indicate that OTU 79 has a closer affinity to OTU's 78, 80, and 81 than it does to OTU's 77, 82, and 83. These relationships differ from those expressed in the distance phenogram (Fig. 7b). It separates OTU 79 from OTU's 77, 82, 83, 80, 81, and 78. The distance phenogram also indicates a more distant relationship between OTU 78 and OTU's 80 and 81. The degree of relationship can be seen in the distance value (1.440) which expresses the level of union of the two OTU's with OTU 78.

255 OTU Phenograms

The correlation phenogram places OTU's 75 and 76 closer to the OTU's of the *H. spinulosus* group than to any of the other parental taxa (Fig. 11). This and the 64 OTU correlation phenogram are the only phenograms in which this association is seen. The distance phenogram, as did the 64, 26, and 9 OTU phenograms, separated these two OTU's from all other parent OTU's. In both the 255 OTU phenograms these OTU's are grouped with hybrids in which one or the other serve as a parent. OTU 82 (2998) is shown to be more closely related to OTU 83 (3009). These two then are joined to OTU's 80 (2737), 81 (2769), and 78 (2611) in this same order. OTU 77 *(H. texensis)* was placed closer to OTU 79 *(H. gooddingii)* in a large cluster in which there were a number of hybrids in which neither OTU 77 or 79 served as a parent. A number of these "misplaced" hybrids are seen on both phenograms and will be considered later.

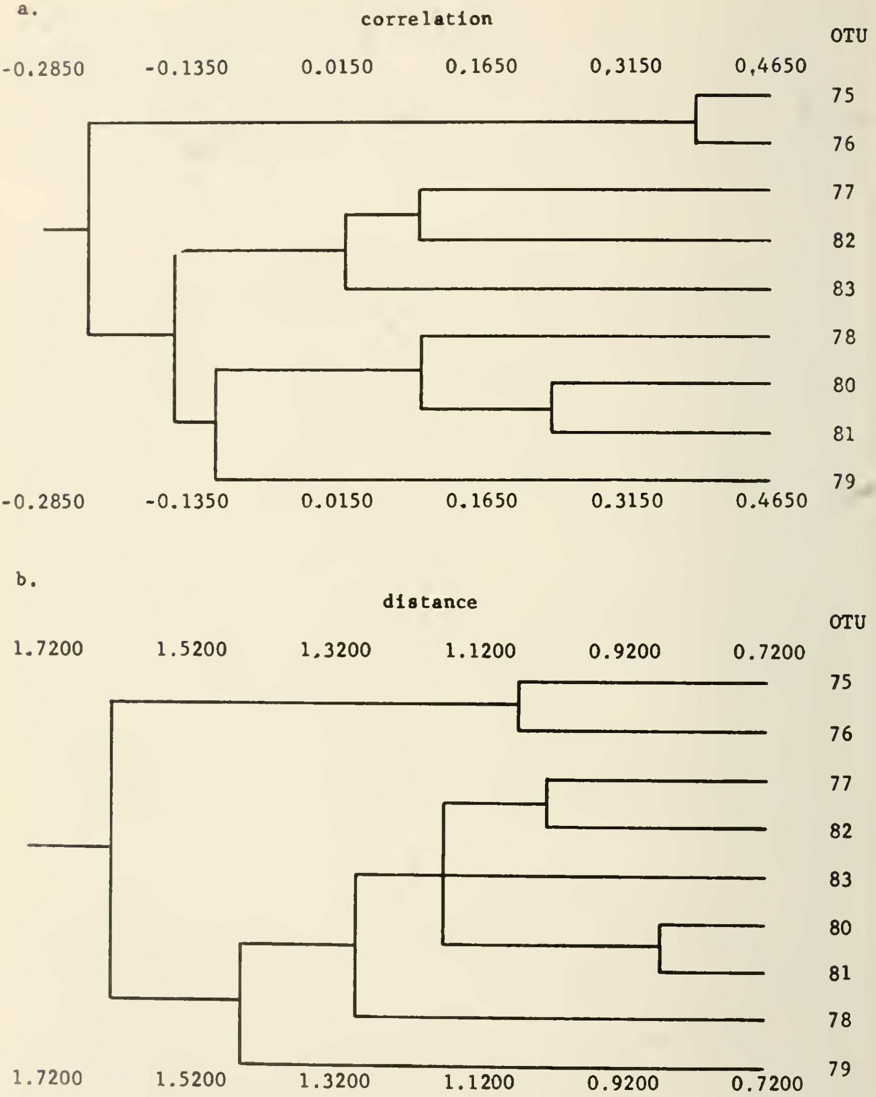


FIG. 7. 9 OTU Correlation and Distance Phenograms. The OTU's represent mean values for the nine parent taxa. Fig. 7a, correlation phenogram; 7b, distance phenogram.

The distance phenogram shows OTU 77 to be more closely related to OTU's 82 and 83. OTU's 80 and 81 are grouped together along with a number of hybrids. OTU 79 *(H. gooddingii)* is shown to be more closely related to four members of the *H. spinulosus* complex than to OTU 78 (2611) which is considered to be a member of the complex (Appendix Fig. 2).

The cophenetic correlation coefficient, expressing the accuracy of the phenogram in reflecting the relationships of the data matrix, was lowest in the correlation phenogram (0.772). The 0.9880 correlation value for the distance phenogram would indicate that the relationships that it expresses were far less distorted.

In comparing the relationships of the parental taxa of the 9 and 26 OTU phenograms it is evident that the distance phenograms are essentially identical (Figs. 6, 7). When you compare the correlation phenograms a single difference is noted. This difference is in the placement of OTU 78 (2611). The 26 OTU phenogram indicates that this OTU has a closer relationship with OTU 82 (2998) and OTU 83 (3009) while the 9 OTU phenogram clusters it initially with OTU's 80 (2737) and 83.

The 64 OTU phenograms (parent and hybrid means) agree in most respects with the 9 and 26 OTU phenograms in the relationships expressed among the parents. Particularly, the correlation phenogram separates OTU's 75, 76, 77, and 79 quite distinctly from OTU's of the *H. spinulosus* complex (Figs. 2, 5, 7a). These latter OTU's, 78, 80, 81, 82, and 83, are all grouped in a single major cluster. The 255 OTU correlation phenogram reveals this same relationship. OTU 78 (2611) was shown to have a closer affinity to OTU's 80 (2737) and 81 (2769) in the 9, 64, and 255 OTU correlation phenograms while the 26 OTU clustering it was closer to OTU's 82 (2998) and 83 (3009) (Fig. 5).

The relationships indicated in the 64 OTU distance phenogram were somewhat different from those seen in the correlation phenogram. OTU 78 (2611) was shown to be closer to OTU 83 (3009). All other OTU's of the *H. spinulosus* complex were placed closer to each other and to OTU 77 *(H. texensis)* than to OTU's 78 or 83. OTU 79 *(H. gooddingii)* was set apart from all OTU's, however, being closer to 77, 78, 80, 81, 82, and 83 than to OTU's 75 and 76. OTU 77 was shown to have a closer affinity to OTU 82 while the correlation phenogram places it in a closer relationship to OTU 79 although the low correlation value indicates that this relationship is more distant than one may see in the phenogram.

THREE-SPACE PROJECTIONS

Three-space projection values were calculated for all of the data matrices. From these values the projection plots shown in Figures 8, 9, 10, and 11 were constructed. The fourth projection plot (255 OTU) shows only the parental taxa. References will be made to this projection but the main coverage of this segment of the study will deal principally with the 9, 26, and 64 OTU projections.

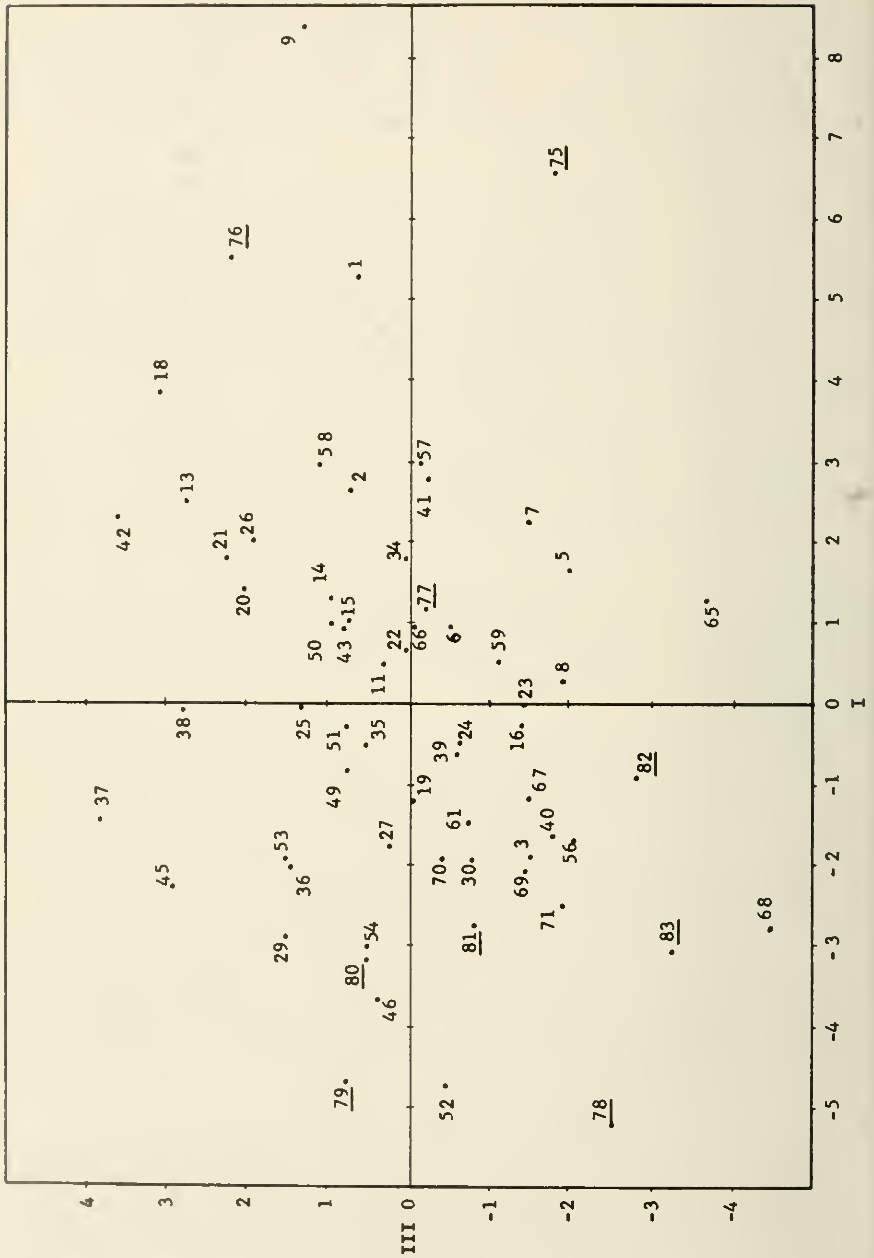
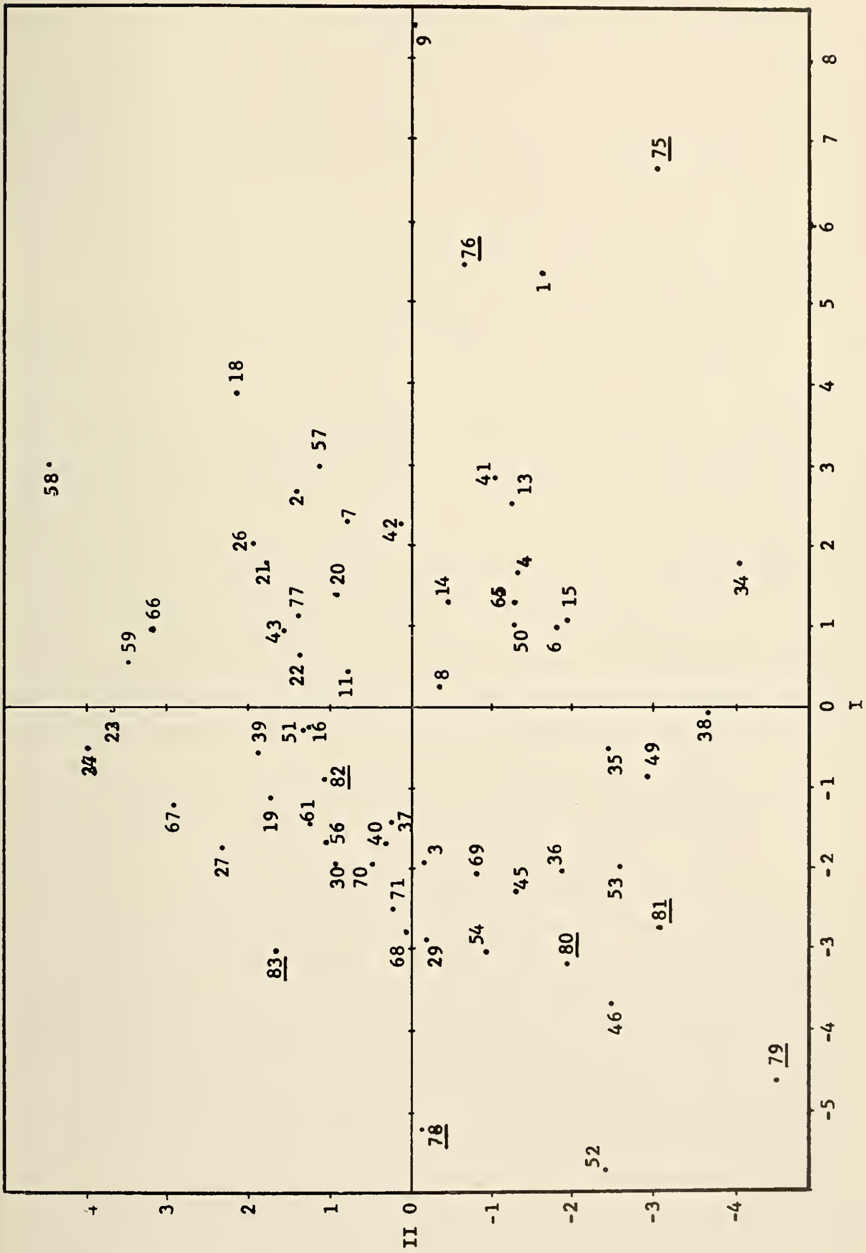


FIG. 8a,b. 64 OTU Three-space Projection Plots. The OTU's represent mean values for the parent and hybrid taxa. The figure should be viewed holding the left page vertical to the right. The parent taxa are underscored.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Placement of the parental taxa in the three-space for the three projections were essentially the same (Figs. 8, 9, 10). There are, however, minor variations in the relative positions of the OTU's between the different projections. OTU's 75 (subsp. *arenarius*), 76 (subsp. *incisifolius*), 78 (2611), and 79 *(H. gooddingii)* are shown to be separated from OTU's 80 (2737), 81 (2769), 82 (2998), and 83 (3009). All four of the latter OTU's are members of the *H. spinulosus complex*. OTU 78 was in every instance placed off by itself and was never included with other members of the complex of which it supposedly is a member. This can easily be seen in Figures 9 and 10.

The 64 OTU projection separates OTU 80 and 81 from 82 and 83 and shows a closer relationship between OTU's 80 and 81 to OTU's 78 and 79 than to OTU 82 and 83. It also places OTU 82 closer to OTU 77 than to OTU's 80 and 81. This placement of OTU's agrees with the relationships expressed in the 26 OTU correlation phenogram (Fig. 5). The hybrids in the 64 OTU projection generally are placed somewhere between the two parents involved in the crosses. In some instances the hybrids were well separated from either parent while others were placed relatively close to one or the other of the parents. Placement of OTU 1 and 9 close to OTU's 75 (subsp. *arenarius*) and 76 (subsp. *incisifolius*) can easily be noted in Figure 8. A complete analysis of the placement of hybrids in the 64 OTU projection will not be attempted here because of the number of OTU's involved. The majority of the relationships shown in this projection can be seen in the Figure 8. Most of the relationships seen in the projection have been pointed out in the discussions of the phenograms.

The placement of the individual plants of the parent taxa is shown in Figure 9. The relations expressed in this projection are perhaps the closest to those in the present classification of these taxa. Placement of the OTU's in the 26 OTU projection is very similar to that of the 64 OTU projection. The projection shows a closer relationship between OTU's 80 (2737), 81 (2769), 82 (2998), and 83 (3009) although OTU 81 is placed some distance from the other three. OTU 83 appears to be located very close to OTU's 80 and 82 (see Fig. 9a). However, if one looks at Figure 9b where the third dimension is considered, it is shown to be separated some distance from OTU's 80 and 82. This distinction is not seen in the 9a OTU projection where it is shown to be quite close to OTU 80. This relationship is also not seen in any of the phenograms.

OTU 77 *(H. texensis)* in each projection was placed closest to OTU's 82 (2998) and 83 (3009). This relationship is seen also in the phenograms in Figures 6 and 7a. The individual plants of *H. texensis* are separated from each other in the 26 OTU projection rather than forming a compact cluster. An explanation for this separation could be that the plants used in scoring and measuring the characters had just begun to flower and also varied considerably in size. Since approximately 40 percent of the characters were measurements of size, the placement of these OTU's could have resulted as they did. In all projections, OTU 77 was noted to have its closest affinity to OTU's of the *H. spinulosus* complex.

OTU 78 (2611) was separated from the other members of the *H. spinulosus* complex (80, 81, 82, 83) in all three projections (Figs. 7, 8, 9). This placement was unexpected as OTU 78 has many features in common with OTU's 82 and 83. The 26 and 64 OTU projections show that this OTU has a closer relationship to OTU 83 (3009). This same affinity was also expressed in the 64 OTU distance phenogram (Fig. 4) while in the correlation phenogram OTU 78 was shown to be more closely related to OTU's 80 (2737) and 81 (2769).

All three-space projections separated OTU 75 (subsp. *arenarius*) and 76 (subsp. *incisifolius*) from all other parental taxa and pointed out also the differences between the two. OTU 77 *(H. texensis)* was placed closest to OTU 75 and 76 in every projection. None of the phenograms indicated this relationship.

To check the degree of agreement between the projected distances calculated using the three centroid axes and the distance matrix of 28 characters, a correlation between the two distance matrices was calculated. A correlation value approaching one (1.0) would indicate how accurately the three-space projection reflects the relationships in the 28-space projection. The correlation values for the three-space projections generated were 0.955, 0.931, 0.904, and 0.988 for the 9, 26, 64, and 255 OTU projections respectively. These values indicate that the projections represented very accurately the 28-space projections.

DISCUSSION AND CONCLUSIONS

The relationships expressed among the nine taxa as a result of the different numerical taxonomic analyses varied. Variable results have been found previously in NT studies in bees by Michener and Sokal (1965) and by Katz and Torres (1965) in *Zinnia*. Heiser *et al.* (1965) found differences in arrangement of OTU's when comparing phenograms generated from standardized and unstandardized data. Rohlf (1963) found variations in placement of mosquitoes in phenograms produced by different methods. The present study gave not only differences between the correlation and distance phenograms but also between the different data matrices used in the study as might be expected. The placement of a specific OTU within a cluster in which other parental taxa of close affinity were grouped changed within the different phenograms.

There was general agreement in the phenograms that OTU's 75 (subsp. *arenarius*) and 76 (subsp. *incisifolius*) are indistinctly separated from the

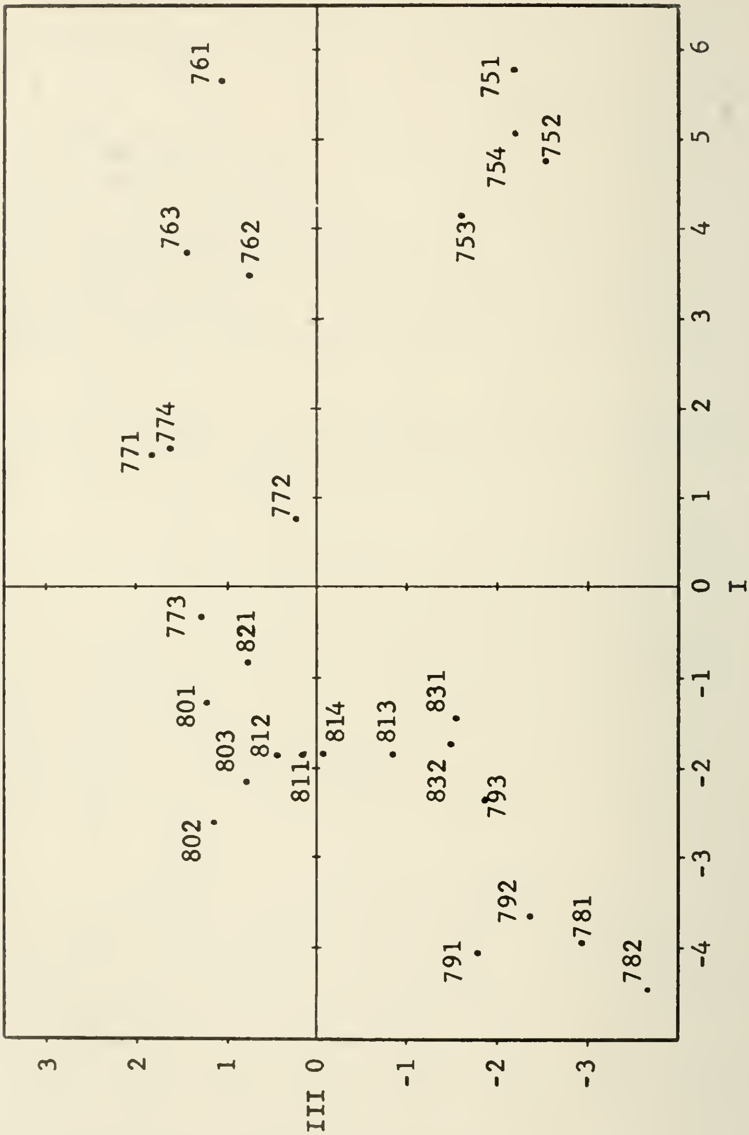
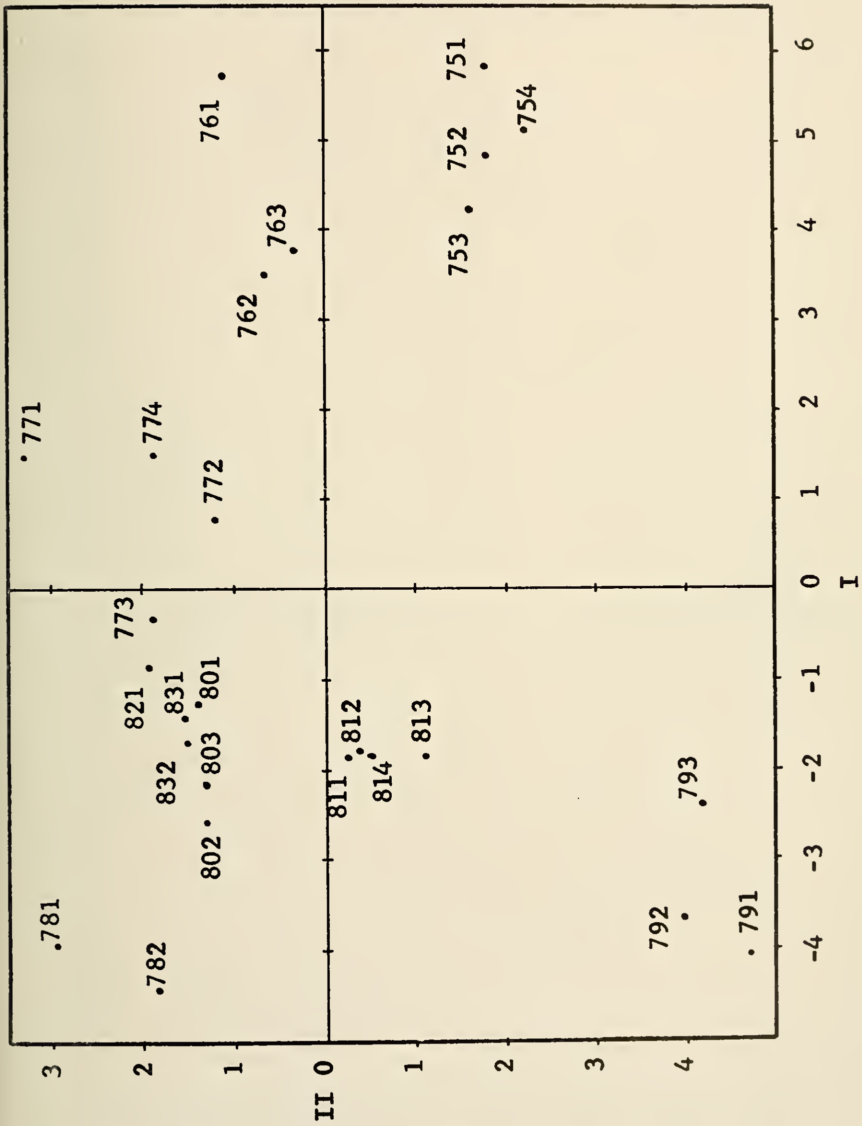


FIG. 9a,b. 26 OTU Three-space Projection Plots. Each OTU represents an individual plant of a parent taxa. View with left page vertical to right.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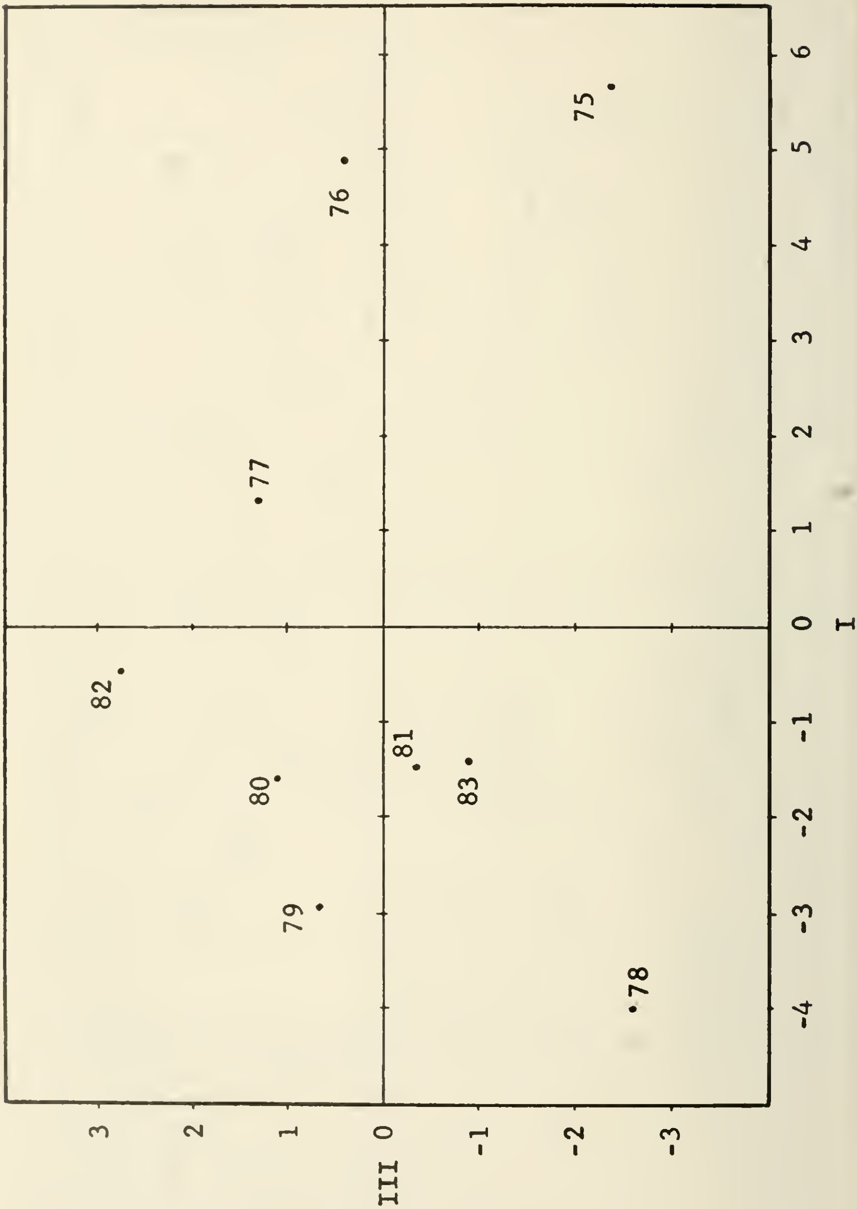
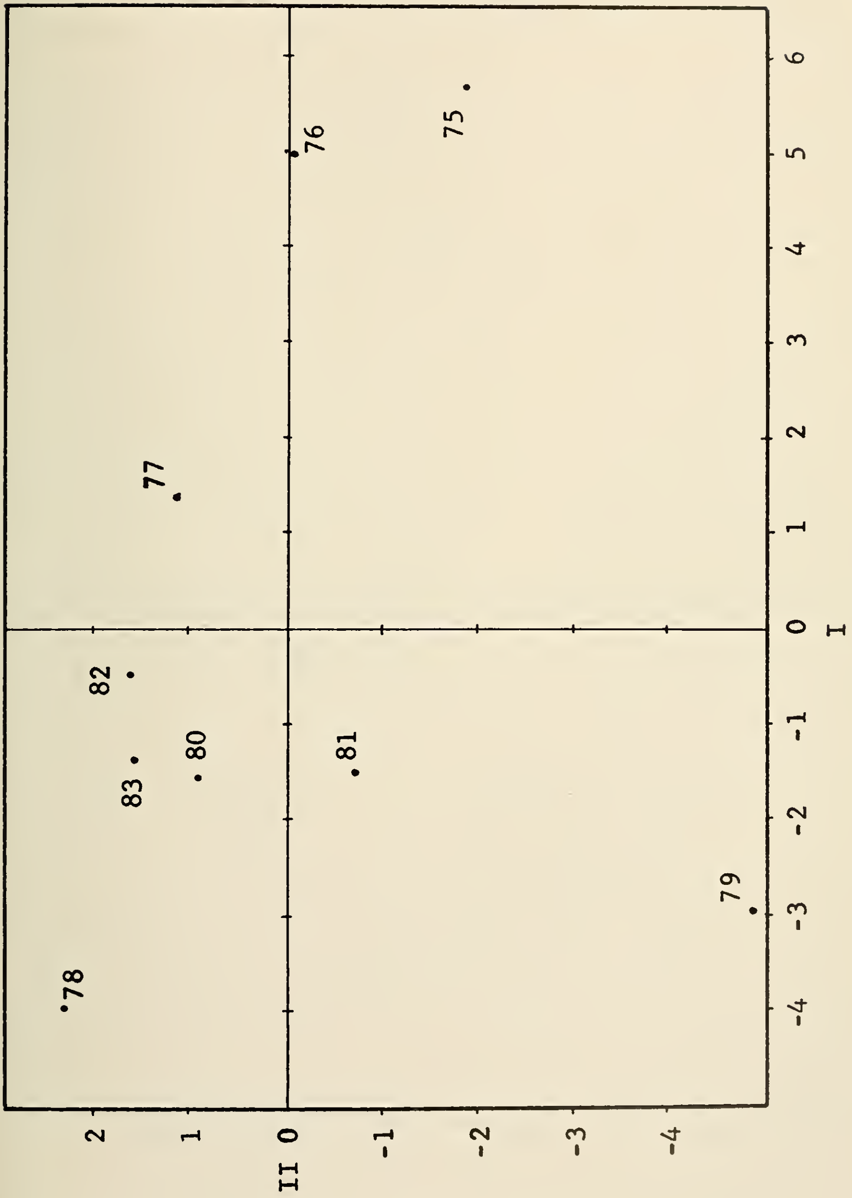


FIG. 10a,b. 9 OTU Three-space Projection Plots. The OTU's represent mean values for the parent taxa. View with left page vertical to right.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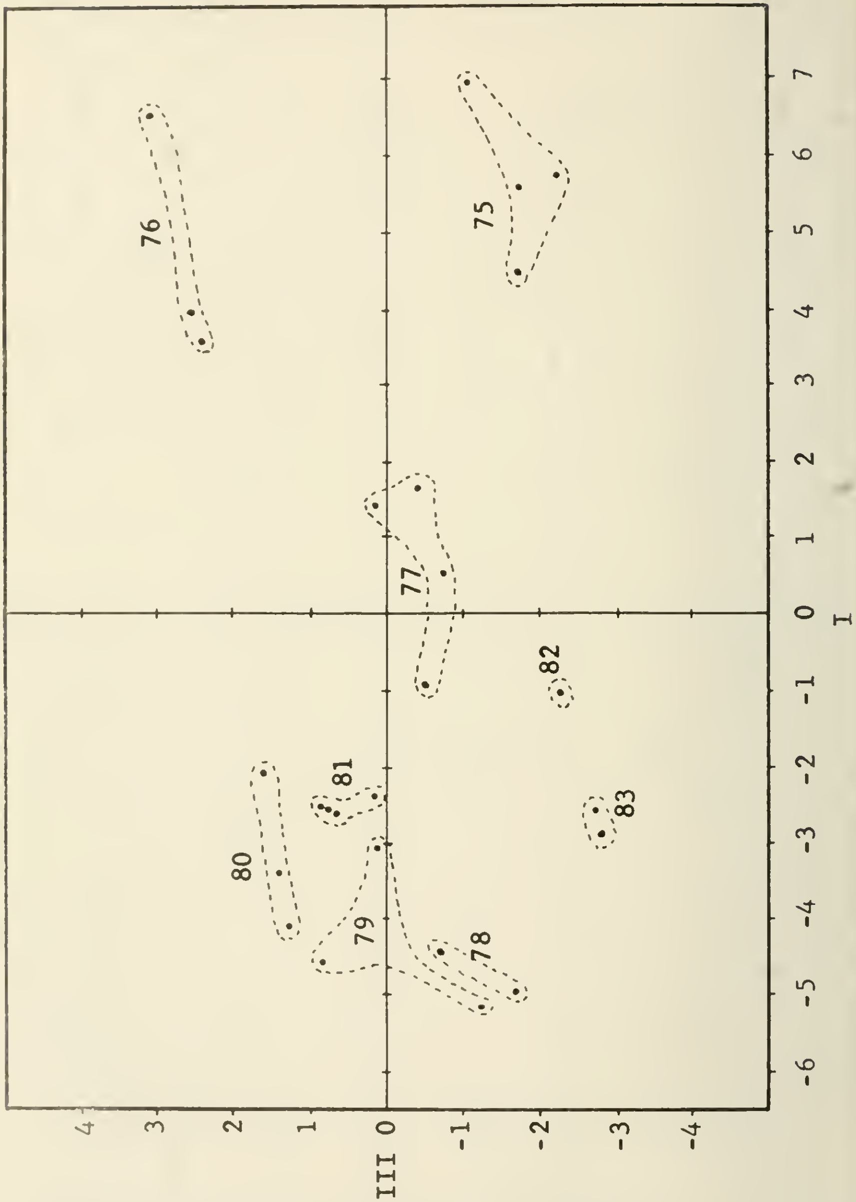
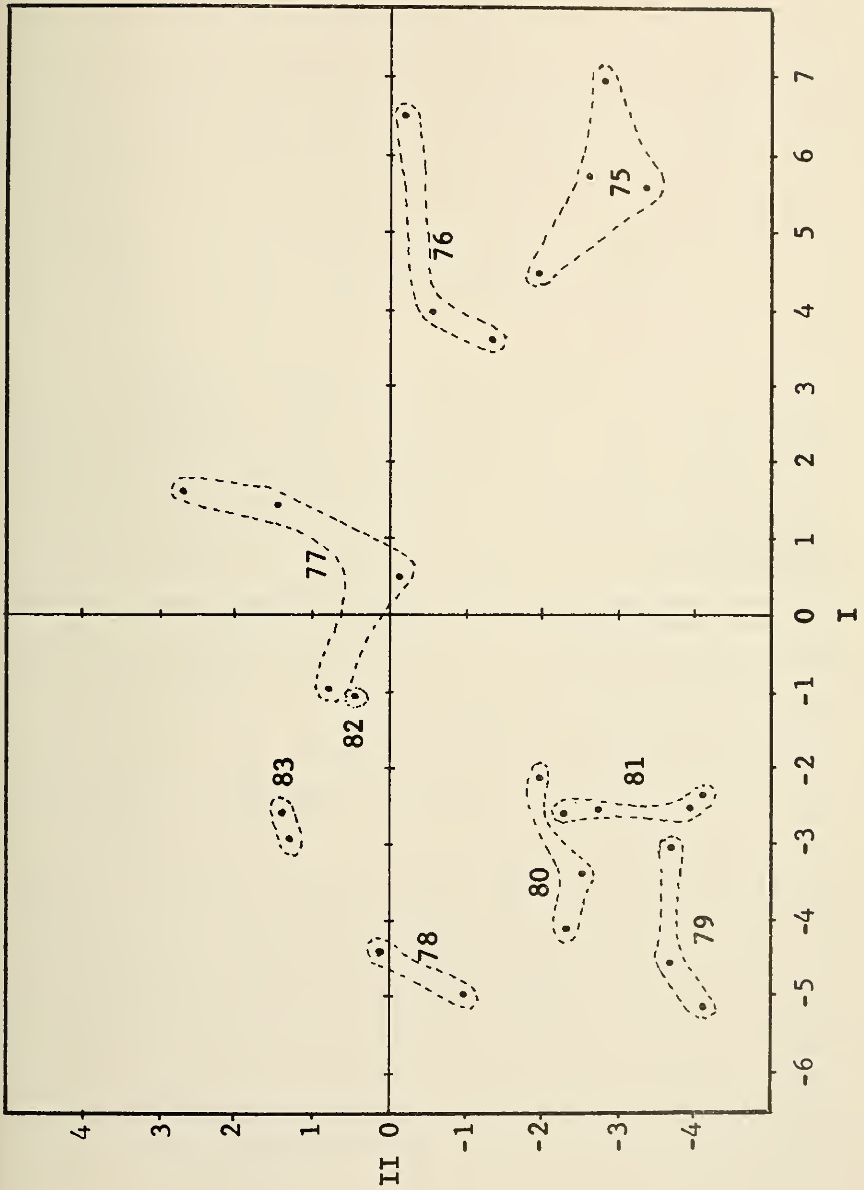


Fig. 11a,b. 255 OTU Three-space Projection Plots of Individual Parent Plants. Only the parent plants were plotted for this projection due to the number of OTU's involved. The OTU's enclosed within the broken lines represent the individual plants for the different parent taxa. View with left page vertical to right.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other taxa. This would indicate that all other OTU's are more closely related to each other than any of them are to OTU's 75 and 76. Two exceptions to this can be seen in the 64 and 255 OTU correlation phenograms where OTU's 75 and 76 are placed closer to members of the *H. spinulosus* complex and shows that OTU's 77 *(H. texensis)* and 79 *(H. gooddingii)* as being the most distantly related to the other taxa. The separation of OTU's 75 and 76 was also noted in all of the three-space projections (Figs. 8, 9, 10, and 11). In three distance phenograms (Figs. 4, 6, 7b) OTU 79 *(H. gooddingii)* is well separated from the two taxa mentioned above as well as to the entire *H. spinulosus* complex (OTU's 80, 81, 82, 83, 78) and from OTU 77 *(H. texensis)*.

The phenograms based on correlation coefficient matrices exhibit a different relationship. The 26 OTU phenogram (Fig. 5) indicates that OTU 79 has its closest affinity to OTU 80 (2737) and 81 (2769) while the 9 OTU phenogram does the same but in addition adds a third taxon, OTU 78 (2611). On the other hand, the 64 OTU phenogram shows that OTU 79 *(H. gooddingii)* has its closest relationship with OTU 77, a result which none of the three-space projections reflects. All three-space projections reveal that OTU 79 is more closely related to certain members of the *H. spinulosus* complex, specifically OTU's 80 (2737) and 81 (2769) (Figs. 8, 9, 10, 11). This same relationship can be seen in the 26 OTU correlation phenogram (Fig. 5). The 255 OTU projection places OTU 79 close to OTU 78 in addition to OTU's 80 and 81 (Fig. 11).

Five of the phenograms show *H. texensis* (OTU 77) to be more closely related to certain members of the *H. spinulosus* complex. The three-space projections also express the same affinities. The 9 OTU correlation (Fig. 7a) and 26 OTU distance (Fig. 6) phenograms place OTU 77 closer to OTU's 82 (2998) and 83 (3009) in this order while the 26 OTU correlation phenogram shows a closeness to OTU 78 (2611) in addition to 82 and 83 (Fig. 5). OTU 77 in the 64 OTU distance phenogram is placed closest to OTU 82 and then joined with OTU's 80 (2737) and 81 (2769). All four projections show that OTU 77 is nearer to OTU's 82 and 83 (Figs. 8, 9, 10, 11).

Comparing the results of the phenograms and three-space projections with the presently accepted taxonomy of the nine different parental taxa, it can be seen that a "loose" agreement occurs. Present taxonomic treatments place OTU's 75 and 76 as subspecies *arenarius* and *incisifolius* of *Haplopappus arenarius*. The numerical analyses presented here seem to reveal these same relationships. The members of *H. spinulosus* complex are shown, in most instances, to be more closely related to each other than to any of the other taxa. *H. texensis,* however, was placed closest to OTU's 82 (2998) and 83 (3009) in the 26, 64, and 255 distance phenograms (Figs. 6, 4, and Appendix Fig. 2). *H. texensis* (OTU 77) is morphologically very distinct from all other taxa used in this study and in the majority of characters is quite different from OTU's 82 and 83. The placement of some individual plants of OTU 77 close to OTU's 82 and 83 in the 26 OTU projection (Fig. 9) and the phenograms mentioned above could be explained by the fact that the character and character states chosen in the study were inadequate to cause a further separation of these particular taxa. As stated earlier, another possibility might be that the individual plants of *H. texensis* that were scored varied considerably in size. Rohlf and Sokal (1965) have shown that phenograms using distance coefficients are more affected by organism size than are correlation phenograms.

The greatest point of disagreement between the results of the numerical analyses and present taxonomy is found in the status and placement of OTU 78 (2611). The latest taxonomic treatment of *Haplopappus* by Hall (1928) would classify this OTU as a subspecies of *H. spinulosus* where the three-space projections generated in the numerical analyses placed it some distance from the other members of the group (Figs. 8, 9, 10, 11). This separation is also shown in some of the distance phenograms (Figs. 6, 7b, and Appendix Fig. 2) which usually place OTU 77 *(H. texensis)* nearer the *H. spinulosus* complex than is OTU 78, although the 64 OTU phenogram does place OTU 78 (2611) close to OTU 83 (3009). Perhaps it should be emphasized again that the distance phenograms (with the exception of the 26 OTU distance phenogram) had higher cophenetic correlation coefficients than those computed for the correlation phenograms. This was also found by Rohlf (1963) in his studies with mosquitoes and by Moss (1966) in his work on the martin mite, *Dermanyssus.*

Pollen fertility percentages of the hybrids did not completely indicate the trends of relationship among the parent taxa that were expressed in the numerical analyses (Table 4). In studies with Solanum, Heiser *et al.* (1965) noted that hybrid fertility did not agree with previous taxonomy or the phenetic relationships given by his numerical analyses. Pollen fertility in this study did show that OTU 78 (2611) in crosses with other members of the *H. spinulosus* complex did produce hybrids with rather high pollen fertility, at least higher than those hybrids in which *H. gooddingii* (OTU 79), *H. texensis* (OTU 77), subsp. *arenarius* (OTU 75) and subsp. *incisifolius* (OTU 76) were the other parents. Hybrids between OTU's 78 and 77 did show a rather high fertility percentage (highest 94.2, average 85.9). If F_1 hybrid fertility, in the absence of detectable structural differences of the chromosomes can be taken as an index of relationship, these two taxa should be considered as being relatively close to each other. This closeness, however, is not expressed in any of the phenograms or projections produced by the numerical analyses. Geographic distance and subspeciation may help explain the high fertility between the two OTU's since OTU 79 is located closest to OTU 78. If one of these OTU's gave rise to the other or each originated from a common ancestor, phenotypic divergence could have occurred without affecting pollen fertility.

Haplopappus gooddingii (OTU 79), in crosses with three members of the *H. spinulosus* (OTU's 80, 81, 82), produced hybrids with pollen stainability above 80.0 percent which would tend to indicate some degree of affinity among the taxa. The 9 and 26 OTU correlation phenograms do show that these OTU's display some relationship which, however, is not close as indicated by the three-space projections (Figs. 7a, 5, 8, 9, 10, 11). The projections instead reveal that OTU 79 is quite distinct from all other taxa.

H. arenarius subsp. *arenarius* and subsp. *incisifolius* in crosses with other taxa show reduced pollen fertility in the resulting hybrids (Table 4). Crosses in which subsp. *incisifolius* served as the pistillate parent showed the highest fertility percentages, particularly in crosses with OTU's 80 (2737) and 82 (2998). This relationship was not seen in the numerical analyses where both OTU's 75 and 76 were consistently placed apart from these two OTU's.

Pollen fertility percentages clearly indicate that OTU's of the *H. spinulosus* complex are related to each other since the percentages are quite high in crosses when members were used as both parents (Table 4). This would seem to indicate that these taxa are in fact related, possibly, more closely to each other than to the remaining parental taxa. The fertility values of OTU's 80, 81, 82, and 83 in crosses with OTU 78 (2611) are high in every instance where hybrids between the taxa were obtained. This seems to agree to some extent with the results expressed in the phenograms and three-space projections.

The high fertility values seen in crosses between *H. texensis* (OTU 77) and OTU's 82 (2998) and 83 (3009) are reflected in the relationships expressed in the 9 and 26 OTU correlation and distance phenograms (Figs. 5, 6, 7a, 7b). The distant relationship seen in the three-space projections between OTU's 77 and 79 is likewise expressed in pollen stainability (61.5 highest, 61.0 average) (see Table 4). Low percentages were also recorded in *H. texensis* × 2737 hybrids (where *H. texensis* was the pistillate parent); however, higher percentages were seen in the reciprocal cross.

Chiasma frequencies were highest in crosses where OTU's 76 (subsp. *incisifolius*) and 77 *(H. texensis)* were the pistillate parent and crossed with OTU's 80, 81, 82 and the reciprocal cross with OTU 83. The frequencies in these hybrids were above five chiasmata per cell (Table 3). Only six other hybrid combinations showed frequencies that were this high; *arenarius* × 2998, 2769 × *arenarius, texensis* × 2611, 2737 × 2769, 2769 × 3009, and *H.*

gooddingii × 2737. These values seem to contradict the relationships expressed in the numerical analyses and pollen fertility data. For example, subsp. *arenarius* and 2998 are the two taxa with the greatest geographic separation of any taxa used in the study. If we assume that a high chiasma frequency in their F_1 hybrid indicates greatest chromosomal homology, the cross would be hard to explain as neither the pollen fertility percentages nor the numerical studies indicate a close affinity between these two OTU's. Some of the other hybrids in which five chiasma per cell occurred would not present the same problem as the one just mentioned. 2737 × 2769 and possibly 2769 × 3009 would be expected to have high values as 2737 and 2769 are quite similar to each other and occur very close to one another while 3009 comes from a locality not too distant from the two Mexican taxa.

Chiasma frequencies in a general way tended to support the present classification of the nine taxa considered although in many cases the values were not as indicative of the possible relationships as might have been expected. As all taxa were members of the same genus and all had the same chromosome number, chiasma frequencies may not be affected by those small chromosomal changes which could effect phenetic characters and pollen fertility in crosses among taxa.

If the results obtained from the cytological, hybridization, and numerical studies were combined, a more accurate classification and thus an expression of relationship between the nine taxa should result. Moss (1966) recommends that a combination of different numerical methods would produce the most acceptable classification of *Dermanyssus* along with other data. An even greater insight into the affinities among the taxa would have been possible if artificial tetraploids had been obtained in some of the hybrids. By studying the pairing of chromosomes at meiosis of these tetraploids it could have been determined whether preferential pairing occurred. In polyploids where a chromosome has more than one pairing partner, it would tend to pair preferentially with the chromosome with which it has the greatest homology (Darlington, 1937). Had preferential pairing occurred, one could then assume that there were enough differences in the members of the genomes of the different taxa to keep all four chromosomes of the same type from pairing to form multivalent configurations. This then could be taken as an indication of homology or the lack of it among the different genomes.

A single hybrid in this study was found to be a tetraploid (2611 × subsp. *incisifolius*). Only a single bud was taken from the plant and since that one, none have been produced to allow further investigation. The cells that were examined in the tetraploid revealed that the majority of the configurations were bivalents. This would suggest that preferential pairing had occurred, in other words that 2611-chromosomes had paired with each other and *incisifolius* chromosomes with each other. The presence of multivalents would have indicated that the two genomes were homologous in enough regions to allow partial pairing of the four chromosomes. As multivalents were not found, at least in the few cells examined, one could assume that even though the karyotypes of both parents show no apparent differences, some changes have occurred between the chromosomes of the different taxa.

The four phenograms in which hybrid OTU's were considered reveal that some hybrid combinations are placed closer to other parent OTU's than to those OTU's used as parents in the crosses (Figs. 3, 4, and Appendix Figs. 1, 2). Heiser *et al.* (1965) found this in the numerical studies on the genus *Solanum*. There were several to many hybrids in the present study that could be classed as "misplaced." In this study both correlation phenograms (Fig. 3, Appendix Fig. 1) show large clusters of hybrids that were placed closer to nonparental taxa than to parent taxa. These placements would indicate that certain of these hybrids are phenotypically closer to nonparental taxa. As phenetic characters are the direct or indirect expression of an organism's genotype, the more closely related genotypes would occur on the phenograms in small compact clusters. Hybrids between parents of dissimilar genotypes should be farthest removed from the parents. If, however, the characters expressed by one of the parents were dominant over those of the other then the hybrids would occur closest to that parent. This seems to have been the case with some of the parental taxa, although the phenograms showed variation in the placement of some hybrid combinations. These changes in placement that are shown in the 64 and 255 OTU phenograms may have resulted from the increased number of OTU's in the data which would make slight changes in the correlation and distance matrices from which the phenograms were generated. Certain characters may be more important in certain species than in others, and, as Heiser *et al.* (1965) points out, the importance or significance of characters may change from one species to another.

If by chance a hybrid between two distinct species could attain reproductive stability by means of polyploidy, it would be possible to classify this hybrid as a species entirely different from either parent. A situation of this type considered strictly on phenetic criteria would be misleading and points to the need for other types of studies in determining classifications. Crossing experiments, cytological and cytogenetic analyses in addition to ecological data would allow an investigator to obtain a more accurate picture of the true affinities among taxa.

If all the evidence obtained in the study were considered in proposing a classification of the nine taxa the following would result. *Haplopappus arenarius, H. texensis, H. gooddingii,* and taxon 2611 would be given species designations. *H. arenarius* would be subdivided into two subspecies, OTU 75 as subsp. *arenarius* and OTU 76 as subsp. *incisifolius*. The remaining four

OTU's (80, 81, 82, 83) could be designated as subspecies of *H. spinulosus.*

Numerical taxonomic analyses should be considered as one of many approaches in attempting to determine accurate classification of organisms. This viewpoint has been expressed most recently by Gilmartin (1967). She stated that "N.T. studies can be included in one's classification along with the results of classical methods." Irwin and Rogers (1967) in their monographic study of *Cassia,* section *Apoucouita,* utilized in addition to numerical analyses "interpretations" in establishing the classification of their material. There is no doubt that NT studies can be useful in different situations in arriving at clearer relationships between taxa, particularly in those instances which have not been made clear by other means and where large amounts of data are being considered.

The different relationships expressed in the numerical taxonomic treatments require workers to use personal judgement in determining "cutoff" lines for the different taxonomic levels. In groups of organisms that are not well known or where no previous work on them exists, these lines could be placed at different locations by different taxonomists or systematists. The variable relationships expressed by the different NT analyses could be of help to systematists in proposing a variety of possible affinities to assist in determining ultimate relationships.

There will be instances where the immense task of securing data for the numerous characters required would not produce results that would be of any value to the problem at hand. At present the number of applications of numerical taxonomic studies to different organisms is limited and in those cases where it has been applied it has contributed little to the classifications already established by other means. The study of Moss (1966) on the genus *Dermanyssus* will have to stand the test of time and other workers since his was the first critical investigation of these organisms.

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THE UNIVERSITY OF KANSAS
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COMPARATIVE GROSS MORPHOLOGY OF SPERMATOZOA OF TWO FAMILIES OF NORTH AMERICAN BATS

By

G. Lawrence Forman

Vol. XLVII Pages 901-928 March 26, 1968 No. 16

ANNOUNCEMENT

The *University of Kansas Science Bulletin* (continuation of the *Kansas University Quarterly*) is issued in part at irregular intervals. Each volume contains 300 to 700 pages of reading matter, with necessary illustrations. Exchanges with other institutions and learned societies everywhere are solicited. All *exchanges* should be addressed to

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THE UNIVERSITY OF KANSAS
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Comparative Gross Morphology of Spermatozoa of Two Families of North American Bats

G. Lawrence Forman
Museum of Natural History
The University of Kansas, Lawrence

ABSTRACT

Spermatozoa of 24 species of North American bats representing two families were studied and compared. All genera and most species examined of the family Vespertilionidae have morphologically distinct sperm, whereas members of the family Phyllostomatidae were less distinctive at the generic and specific levels. Methods of preparation and observation of spermatozoa are described, spermatozoa of included species illustrated, and phylogenetic relationships of bats discussed.

INTRODUCTION

There has been a trend in recent years to augment "conventional" treatments of the systematic relationships of animals with information from a variety of sources. In mammalogy, for example, studies of unique hard parts such as bacula and hyoid bones, immunobiologic comparisons, surveys of karyotypes, and analyses of sound patterns, among others, have appeared. This paper reports the gross spermatozoan morphology of some representative phyllostomatid and New World vespertilionid bats. The aims of my pilot study are to establish adequate techniques for staining, observing, and measuring spermatozoa and to determine their possible usefulness in taxonomic investigations of Chiroptera.

The use of sperm morphology as a criterion of taxonomic relationships among mammals is a relatively recent innovation. The first such study was by Friend (1936), who compared the spermatozoa of British Muridae with respect to differences at the specific, generic, and subfamilial levels. Hughes (1964, 1965) compared the spermatozoan morphology of 18 species of marsupials representing five families, and Biggers and DeLamater (1965) observed three distinct morphological types of spermatozoa in several genera of American marsupials. Variation in spermatozoan morphology among mammals was discussed by Bishop and Austin (1957); they suggested that the sperm of each mammalian species probably is distinctive.

There appear to be only four studies dealing with the morphology of chiropteran spermatozoa. Bishop and Austin *(op. cit.)* described the sperm of the greater horseshoe bat, *Rhinolophus ferrumequinum* and Hirth (1960) examined and measured spermatozoa of 10 species representing seven American genera in the family Vespertilionidae. Additionally, Fawcett and Ito (1965) presented detailed descriptions of the ultrastructure of the sperm of two species of vespertilionids, and Wimsatt *et al.* (1966) made similar observations using sperm from *Myotis lucifugus*.

A noteworthy contribution to the use of spermatozoa in establishing systematic and phylogenetic relationships was that of McFarlane (1963), who examined morphological variation in spermatozoa of birds at various taxonomic levels from species to order. Also, he analyzed geographic variation in spermatozoa of the red-winged blackbird, *Agelaius phoeniceus*.

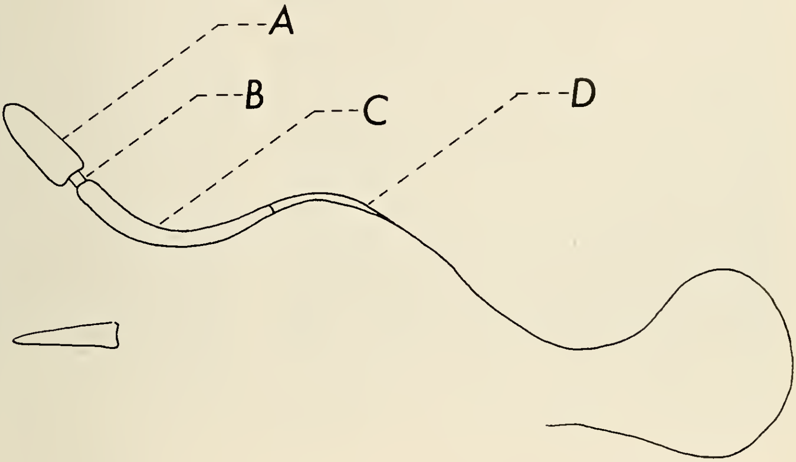
Because of the relatively few species examined and small sample sizes studied, the present report must be regarded as preliminary. Measurements of spermatozoa presented here conflict in several instances with those reported by Hirth (1960). I can only suggest that differences in methods of fixation and staining of tissues account for the differences. Hirth used 10 per cent formalin as a fixative, whereas I used a rapid-fixing propionic acid-alcohol preparation.

MATERIALS AND METHODS

Sperm preparations of bats representing 5 genera and 12 species of the family Vespertilionidae and 6 genera and 8 species of the family Phyllostomatidae were examined. Testes were removed initially from freshly killed males and placed in a fixing solution composed of the following materials: 2 parts 100 per cent methyl alcohol; 4 parts 95 per cent ethyl alcohol; 1 part acetone; 2 parts chloroform; and 1 part 100 per cent propionic acid. In most cases, specimens from which testes were removed are deposited in the Museum of Natural History at The University of Kansas (KU); a few are in the U.S. National Museum (USNM). In some instances, testes were taken from individuals of well-known species that were not subsequently preserved as museum specimens.

In addition to those species listed herein, testes of 15 other species of vespertilionids and phyllostomatids were examined that yielded no spermatozoa or in which only immature spermatozoa were found (epididymal material, from which most mature spermatozoa were obtained, was not preserved with some of the testes, hence the specimens frequently yielded only immature stages).

Sperm preparations were made by placing a short section of tubule on a slide with one drop of specially prepared lactophenol-cotton blue stain. This preparation stained both nuclear material and cytoplasmic matrix and required only a few minutes for penetration. The tubule fragment was teased apart so as to allow spermatozoa to enter the staining medium. A cover slip then was placed over the material, and the edges sealed with balsam.



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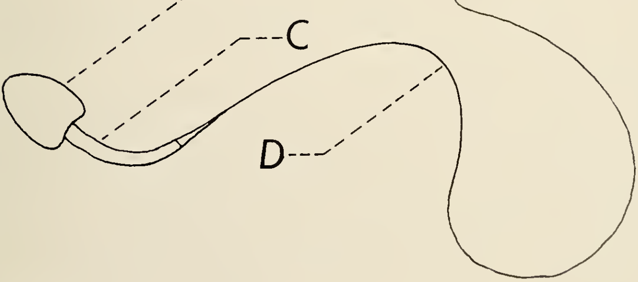

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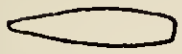

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Fig. 1. A generalized vespertilionid sperm. A, head; B, neck; C, midpiece; D, tail.

Fig. 2. Head of vespertilionid sperm in lateral view.

Fig. 3. A generalized phyllostomatid sperm. A, head; C, midpiece; D, tail. A well defined neck region is absent in spermatozoa of most phyllostomatids.

Fig. 4. Head of phyllostomatid sperm in lateral view.

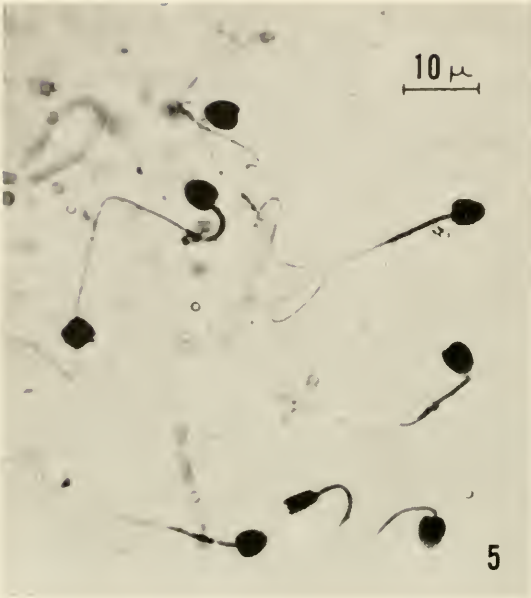


FIG. 5. Photograph of spermatozoa of *Carollia castanea.* One micron equals 1.082 mm on photograph.

FIG. 6. Photograph of spermatozoa of *Myotis grisescens.* Scale as in Fig. 5.

All observations were made using a Leitz Ortholux Research Microscope at a magnification of 1460. Measurements were taken from photographs (Figs. 5, 6) enlarged to 3½ by 4½ inches from 35 mm film. At this magnitude, parallel lines spaced at intervals of .01 mm on a microscope slide represented an enlargement to 10.82 mm (one μ on a microscope slide=1.082 mm on all photographs). Measurements were taken by means of dial calipers calibrated in millimeters, and the measurements were subsequently converted to microns in the following accounts.

Drawings of spermatozoa incorporate average measurements for structures depicted and were made to a scale on which 1 μ=4 mm. The terms "dorsal" and "ventral" refer to the flattened facies of the head and midpiece, whereas "lateral" refers to the narrow sides of the sperm (Figs. 2, 4). Length of head was measured from the greatest extent of the apex to the posterior extremity. An acrosome was not observed in most spermatozoa; consequently, all measurements of the head are of nuclear material only. Width of the head was measured as the distance between lateral extremities as observed in dorsal or ventral view. Depth of the head is the measurement of the greatest extent of the posterior margin in lateral view. Width of midpiece was measured at the anterior extremity in dorsal or ventral view. With reference to head structure, as viewed dorsally or ventrally, an "asymmetrical" base is one in which one side of the head extends farther posteriorly than does the other; a "symmetrical" base has equal posterior limits on either side of the neck.

Tails of spermatozoa frequently were broken during preparation of slides. In the accounts that follow, descriptions and measurements of tails are included only when tails were known to be complete.

ACCOUNTS OF SPECIES

Family **PHYLLOSTOMATIDAE**

This New World family, comprised of seven subfamilies, is more or less restricted to the tropics. More than 40 genera, with a diverse array of species, are represented by insect-, nectar-, and fruit-eating types.

The head of the spermatozoa of phyllostomatids (Fig. 3) is rounded with broadly convex and symmetrical sides. The base may be symmetrical or asymmetrical, and usually is concave. An acrosome was sometimes noted and in a few instances, an extremely short, broad neck was observed. A short, posteriorly-tapering midpiece that is always considerably narrower than the head is centrally attached to the head or neck. The tail is long, narrow throughout its length, and terminates in a narrow endpiece.

All specimens listed as examined are from Mexico, Central America, or the Lesser Antilles.

Subfamily **Phyllostomatinae**

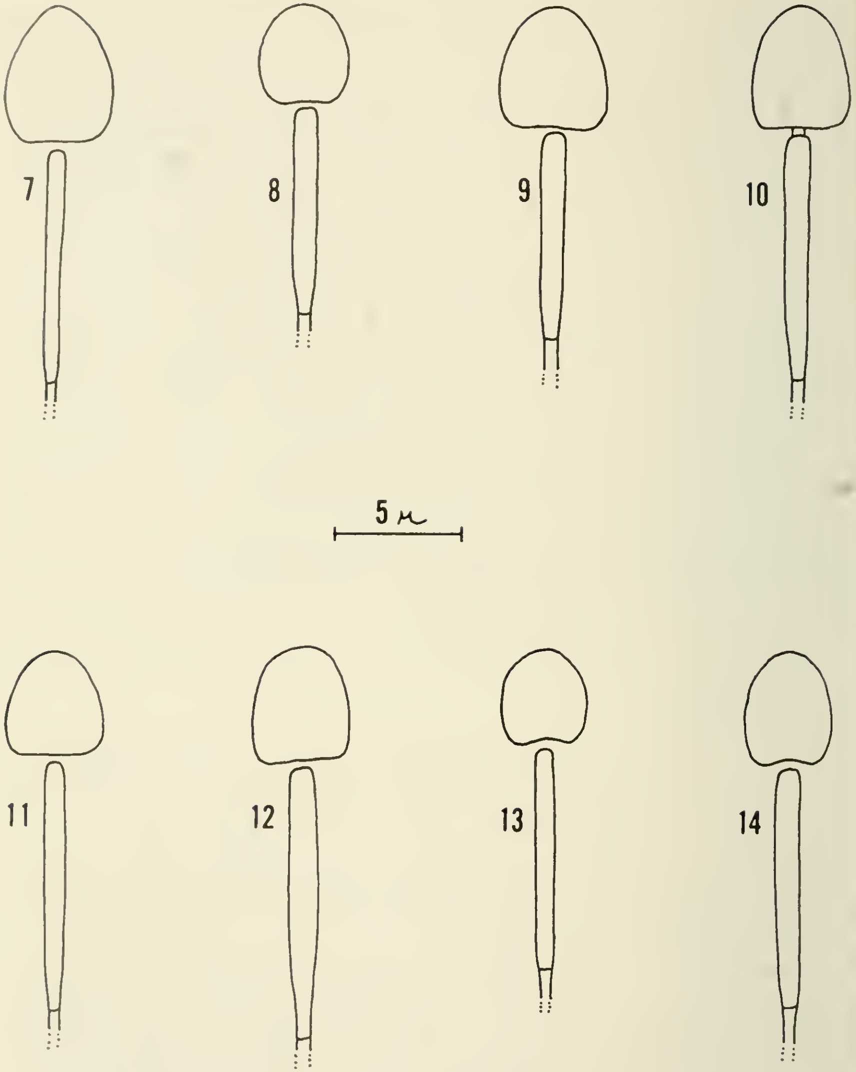
Phyllostomus discolor Wagner, 1843

Fig. 7

MATERIAL EXAMINED. Two specimens of *Phyllostomus discolor verrucosus* from Nicaragua (KU 97474, 97479).

DESCRIPTION. *Head* (based on 20 spermatozoa) base symmetrical (commonest) or asymmetrical; generally large and apex more pointed compared to other phyllostomatids (Fig. 7); longer (by an average of 0.35) than that of any other species studied within the family; length 5.36 (5.27-5.45), width 4.18 (4.11-4.34), depth 1.04 (1.02-1.09). *Neck* not observed although a structure distinct from the midpiece may exist in this species. *Midpiece* (based on 10 spermatozoa) narrow, nonhelical, having little or no tapering posteriorly; an axial filament observed; length 9.06 (8.70-9.35), breadth 0.61 (0.61).

REMARKS. The spermatozoa of *Phyllostomus discolor* are most easily distinguished from those of other phyllostomatids examined on the basis of length of the head, which exceeds that of all other species studied. Also, the head is broad (broader than other phyllostomatids studied except *Anoura*).



FIGS. 7-14. Sperm of some phyllostomatid bats. Fig. 7. *Phyllostomus discolor.* Fig. 8. *Glossophaga soricina.* Fig. 9. *Anoura cultrata.* Fig. 10. *Carollia castanea.* Fig. 11. *Sturnira lilium.* Fig. 12. *S. ludovici.* Fig. 13. *Artibeus jamaicensis.* Fig. 14. *A. lituratus.*

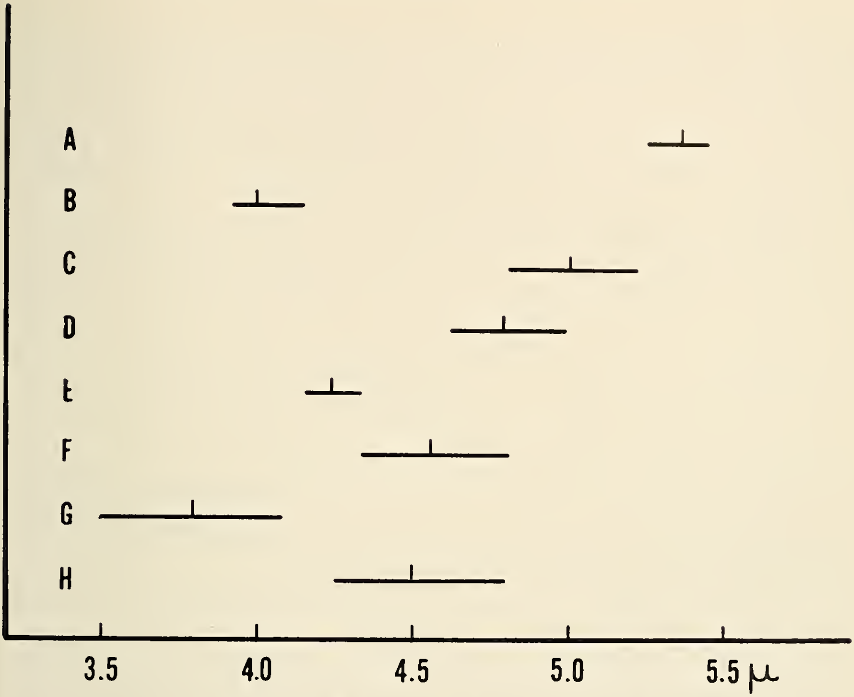


Fig. 15. Comparative head lengths of spermatozoa of some phyllostomatid bats. A, *Phyllostomus discolor;* B, *Glossophaga soricina;* C, *Anoura cultrata;* D, *Carollia castanea;* E, *Sturnira lilium;* F, *S. ludovici;* G, *Artibeus jamaicensis;* H, *A. lituratus.* The observed range is indicated by a horizontal line; a vertical line indicates the mean.

Subfamily **Glossophaginae**

Glossophaga soricina (Pallas, 1766)

Fig. 8

Material Examined. Four specimes of *Glossophaga soricina leachii* from Chiapas (KU 102318, 102320, 102343, 102354).

Description. *Head* (based on 20 spermatozoa) shape and proportions similar to those of several other species of phyllostomatids in that the apex is blunt, base concave, and structure generally rounded; an acrosome was seen on several specimens; head tapers (in lateral view) to a point anteriorly but less sharply posteriorly; length 4.00 (3.93-4.15), width 3.52 (3.33-3.60), depth 0.90 (0.88-0.92). *Neck* not observed in this species. *Midpiece* (based on 8 spermatozoa) broad and of nearly uniform width throughout, short in comparison with other phyllostomatids; granular in character but no true spiral structure identified; length 7.89 (7.67-8.04), width 0.96 (0.92-1.02).

Remarks. Little variation was noted in the spermatozoa of *G. soricina.* They resemble those of *Artibeus jamaicensis* in general appearance, those of

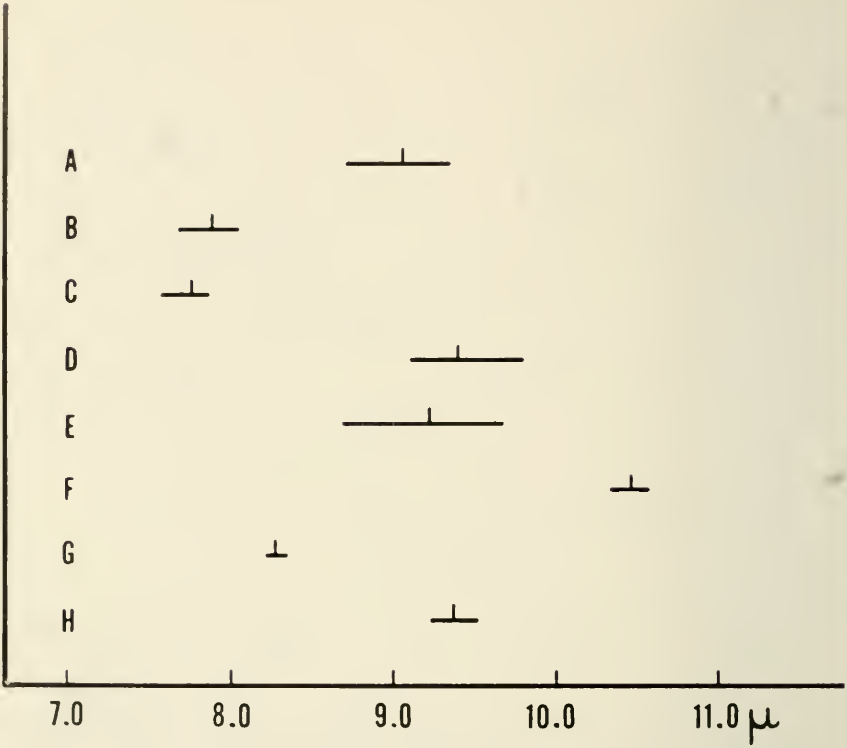


FIG. 16. Comparative midpiece lengths of spermatozoa of some phyllostomatid bats. A, *Phyllostomus discolor;* B, *Glossophaga soricina;* C, *Anoura cultrata;* D, *Carollia castanea;* E, *Sturnira lilium;* F, *S. ludovici;* G, *Artibeus jamaicensis;* H, *A. lituratus.* The observed range is indicated by a horizontal line; a vertical line indicates the mean.

Anoura cultrata in length of midpiece, and those of *Carollia castanea* in ratio of length of head to length of midpiece.

Anoura cultrata Handley, 1960

Fig. 9

MATERIAL EXAMINED. Two specimens from Panama (USNM 337991 and one uncatalogued).

DESCRIPTION. *Head* (based on 20 spermatozoa) broadest in basal region, bluntly rounded at apex; base slightly concave owing to presence of shallow depression at point of attachment to midpiece; head rounded, its breadth approximately seven-eighths of length; dorsoventral flattening evident in that depth of head decreases anteriorly; length 5.01 (4.81-5.22), width 4.35 (4.16-4.57), depth 1.14 (1.02-1.20). *Neck* not observed in spermatozoa of *A. cultrata. Midpiece* (based on 6 spermatozoa) short when compared to length of tail; may be spiraled (resolution not fine enough to verify, but characteristic patches of granulation spaced at regular intervals observed in some); width uniform throughout; length 7.78 (7.58-7.86), width 0.76 (0.69-0.83). *Tail* (measurements based on 2 spermatozoa) about average length for phyllostomatids examined, 50.09 (49.58-50.60); does not taper.

Remarks. The spermatozoa of *Anoura cultrata* demonstrate intergeneric distinctness within the subfamily Glossophaginae when compared with those of *Glossophaga soricina.* The head is broader in *A. cultrata* than in *G. soricina,* the ratio of length to breadth being 1.15 as opposed to 1.28 in *soricina.*

Subfamily **Carolliinae**

Carollia castanea H. Allen, 1890

Fig. 10

Material Examined. Three specimens of *Carollia castanea* from Panama (USNM uncatalogued).

Description. *Head* (based on 20 spermatozoa) rounded, somewhat heart-shaped; base concave and symmetrical; apex broadly rounded; base narrows in lateral view at point of junction with midpiece (Fig. 10); length 4.79 (4.62-4.99), width 3.95 (3.84-4.07), depth 1.13 (1.02-1.20). *Neck* short but distinct, evident in dorsal and ventral views; length less than 0.5 in all specimens examined. *Midpiece* (based on 10 spermatozoa) at distinct angle (rather than parallel) to base of head anterior end (Fig. 10); short, tapering only slightly posteriorly; spiraled in appearance in that granular patches occur at regular intervals throughout length; length 9.4 (9.1-9.8), width 0.88 (0.83-0.92). *Tail* of uniform width, moderately long; length 49.5 (48.6-50.3) in 2 spermatozoa.

Remarks. The head of the sperm of *Carollia castanea* is somewhat longer and narrower than that of *Sturnira* but is shorter than those of *Anoura* and *Phyllostomus.* A spiraled midpiece was observed, confirming the existence of such a structure in at least one subfamily of the Phyllostomatidae.

Subfamily **Sturnirinae**

Sturnira lilium (E. Geoffroy St.-Hilaire, 1810)

Fig. 11

Material Examined. Two specimens of *Sturnira lilium parvidens* from Chiapas (KU 102414, 102425).

Description. *Head* (based on 20 spermatozoa) short and broad; apex extremely blunt; base not concave, the posterior corners (dorsal and ventral views) rounded in contrast to pointed condition observed in other phyllostomatid genera; in lateral view (as in other phyllostomatid spermatozoa), pointed at apex, tapering slightly posteriorly; length 4.24 (4.16-4.34), width 3.81 (3.79-3.88), depth 1.17 (1.11-1.20). *Neck* region not well defined in this species. *Midpiece* (based on 2 spermatozoa) nonhelical, width nearly uniform throughout; ratio of length of midpiece to length of head 2.3 (less than 2.2 in other phyllostomatids studied); no axial filament observed but assumed to be present; length 9.21 (8.73-9.68), width 0.82 (0.79-0.88).

REMARKS. The general morphology of the sperm of *S. lilium* is typically phyllostomatid in that the head is broad, oval, and flattened, and the midpiece is narrow, short, and apparently lacks a distinct neck. Two characters distinguish this species (and *Sturnira ludovici*) from other members of the family: lack of concavity in the base of the head, and the exceptional length of the midpiece in relation to length of the head. The taxonomic significance of these characters is discussed later.

Sturnira ludovici Anthony, 1924

Fig. 12

MATERIAL EXAMINED. Eight specimens of *Sturnira ludovici ludovici* from Panama (USNM uncatalogued).

DESCRIPTION. *Head* (based on 20 spermatozoa) much as in *S. lilium,* differing only in proportions; apex blunt; no concavity in base; lateral extremities rounded; larger (average measurements) than in *S. lilium*; length 4.56 (4.34-4.81), width 3.75 (3.60-3.88), depth 1.20 (1.11-1.29). *Neck* not discernible. *Midpiece* (based on 10 spermatozoa) broad, nonhelical, long (exceeds in length that of all other phyllostomatids studied); variation in length slight (less than .25 in 10 measurements); length 10.46 (10.35-10.58), width 0.99 (0.92-1.02).

REMARKS. The spermatozoa of *S. ludovici* are similar to those of *S. lilium* in gross morphology, but may be distinguished on the basis of length of head and length of midpiece. Length of the head in *lilium* does not exceed 4.34, whereas length is never less than 4.34 in *ludovici*. The length of midpiece is greater than 10.0 in *S. ludovici,* less than 10.0 in *lilium*.

Subfamily **Stenoderminae**

Artibeus jamaicensis Leach, 1821

Fig. 13

MATERIAL EXAMINED. One specimen of *Artibeus jamaicensis praeceps* from Dominica (KU 104871) and one specimen of *Artibeus jamaicensis triomylus* from Nayarit (KU 97013).

DESCRIPTION (based on *A. j. praeceps*). *Head* (based on 20 spermatozoa) short, rounded, nearly circular; marked basal concavity; great width (in relation to length) characteristic; length 3.80 (3.50-4.08), width 3.41 (3.26-

3.67), depth 0.99 (0.90-1.02). *Neck* not distinguishable in the sperm of this species. *Midpiece* (based on 2 spermatozoa) much as in other phyllostomatids; relatively short, narrow, tapering gradually posteriorly near point of junction with tail; length 8.28 (8.23-8.32), width 0.62 (0.55-0.65).

Remarks. The spermatozoa of *A. jamaicensis* resemble those of *Glossophaga soricina,* both in general morphology and in size (compare Figs. 8, 13). The head of *A. jamaicensis* is the shortest among the phyllostomatids studied, being overlapped in measurements only by that of *G. soricina.* Although only two measurements of length of midpiece are available, the sperm of *A. jamaicensis praeceps* may well be distinct among members of the family studied on the basis of this measurement. Midpieces of *A. jamaicensis* spermatozoa seemingly are longer than those of the two glossophagines but shorter than those of other phyllostomatids examined. The ratio of length of the head to length of the midpiece (.89) is similar to that found in *Glossophaga, Anoura,* and *Sturnira.*

Although *A. j. praeceps* is an insular subspecies, and hence potentially reproductively isolated from continental races, its spermatozoa, at least in characters of the head, are essentially as in the mainland *Artibeus jamaicensis triomylus.* Length and breadth of the head in *A. j. triomylus* are 3.93 (3.84-4.08) and 3.44 (3.33-3.60), respectively (based on 10 spermatozoa). These figures indicate remarkable uniformity of sperm structure in two subspecies of *A. jamaicensis.*

Artibeus lituratus (Olfers, 1818)

Fig. 14

Material Examined. Two specimens of *Artibeus lituratus palmarum* from Chiapas (KU 102538, 102580).

Description. *Head* (measurements based on 20 spermatozoa) rounded at apex, little tapered, generally egg-shaped or ovate; base slightly concave, symmetrical or asymmetrical; progressively less flattened dorsoventrally from anterior to posterior; length 4.50 (4.25-4.80), width 3.45 (3.28-3.51), depth 1.41 (1.39-1.43). *Neck* not distinguished in the sperm of this species. *Midpiece* (based on 6 spermatozoa) of nearly uniform width throughout; narrow; little granulated; tapering sharply near junction with tail; length 9.38 (9.24-9.52), width 0.96 (0.92-1.00). *Tail* exceptionally narrow and of uniform width (no apparent tapering); length 71.87 (71.10-72.97) in 6 spermatozoa.

Remarks. The head is relatively long and narrow, generally resembling that of *Phyllostomus discolor.* It is similar in length to the heads of *Sturnira ludovici, S. lilium,* and *Carollia castanea.* The length of the midpiece is similar to that of *Sturnira lilium* and *Carollia castanea.*

The spermatozoa of *A. lituratus* are distinct from those of *A. jamaicensis* in dimensions of both head and midpiece, *jamaicensis* being smaller than *lituratus.* Sperms of both species of *Artibeus* overlap in dimensions with those of several other phyllostomatid genera studied.

Family VESPERTILIONIDAE

In the New World, the family Vespertilionidae includes 14 genera of insect-eating bats in two subfamilies; four of the genera also occur in the Old World. The family is almost world-wide in distribution, absent only from Arctic and Antarctic regions and some oceanic islands.

The spermatozoa of vespertilionids (Fig. 1) are characterized by several distinctive features in comparison with those of phyllostomatids and other mammals. The head is symmetrical with no hooks or ornamentation, has an obtuse apex, is slightly concave at the base, and, in dorsal or ventral view, has nearly parallel sides. The neck is extremely narrow, and centrally attached to the base of the head. The midpiece is usually narrower than the head, and tapers gradually posteriorly. Patterns of striations suggesting a helical structure in the midpiece sometimes were seen. The tail is broad anteriorly and tapers to a narrow endpiece.

Subfamily **Vespertilioninae**

Myotis austroriparius (Rhoads, 1897)

Material Examined. No specimens examined.

Description (after Hirth, 1960:78). *Head* with obtuse apex; broadest in basal region; length 4.6 (4.4-4.9). *Neck* narrow but distinct; no measurements recorded. *Midpiece* helical; broadest anteriorly, tapering gradually posteriorly; length 12.8 (11.5-13.1), width 1.2 (1.0-1.4). *Tail* tapers posteriorly; length 49.0 (47.5-54.0).

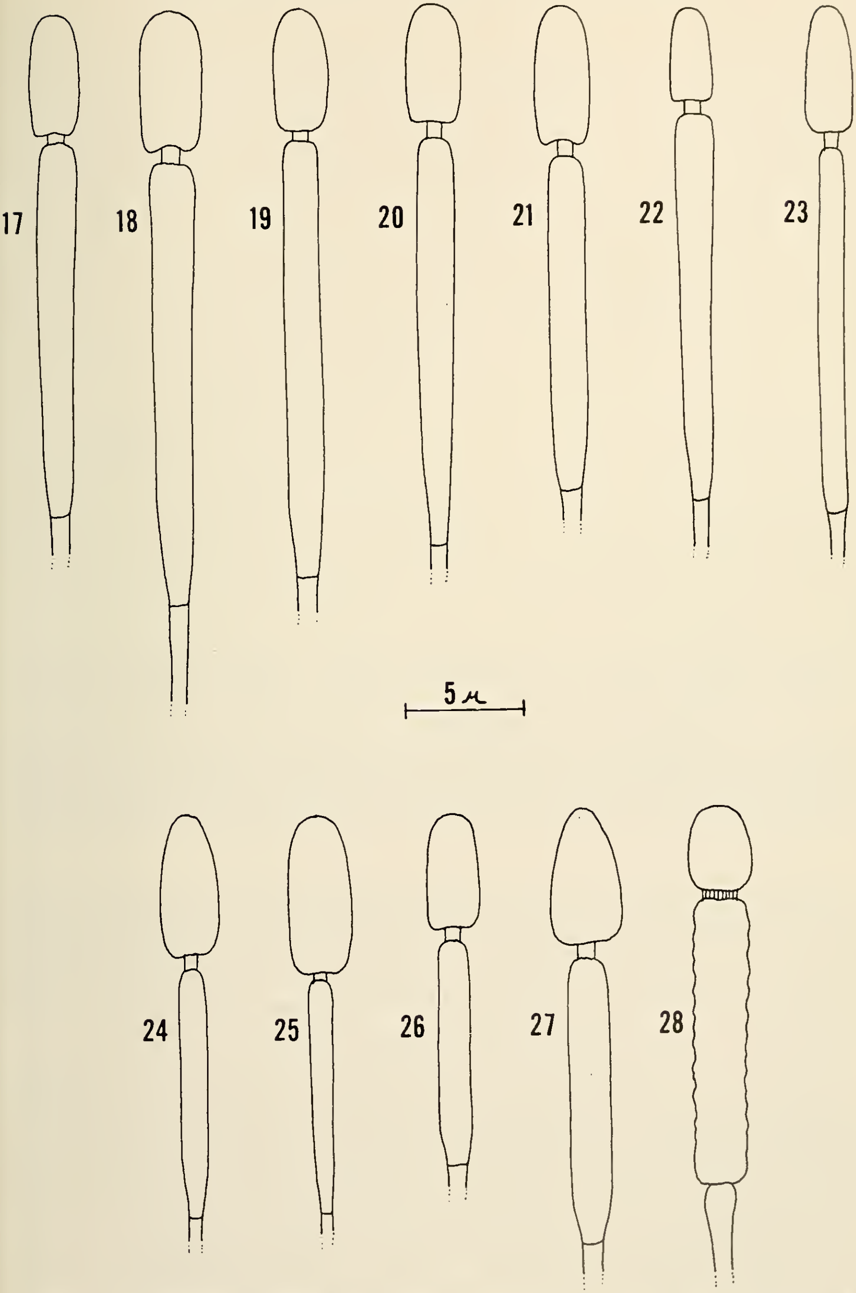
Remarks. In general, the spermatozoa of *Myotis austroriparius* resemble those of other *Myotis.* Judging from Hirth's *(loc. cit.)* measurements, *austroriparius* has a shorter head and midpiece than the other species of the genus that I studied, excepting *M. velifer.*

Myotis evotis (H. Allen, 1864)

Fig. 17

Material Examined. One specimen of *Myotis evotis evotis* from North Dakota (KU 100737).

Description. *Head* (measurements based on 20 spermatozoa) with blunt apex, sides nearly parallel; dorsoventrally flattened, tapering symmetrically anteriorly (in lateral view); characters typical of genus *Myotis* including presence of a slightly concave base; length 4.85 (4.59-5.20), width 2.08 (1.87-2.24), depth 1.40 (1.22-1.50). *Neck* distinct, but most prominent in



FIGS. 17-28. Sperm of some vespertilionid bats. Fig. 17. *Myotis evotis*. Fig. 18. *M. grisescens*. Fig. 19. *M. keenii*. Fig. 20. *M. nigricans*. Fig. 21. *M. sodalis*. Fig. 22. *M. velifer*. Fig. 23. *M. volans*. Fig. 24. *Pipistrellus subflavus*. Fig. 25. *Eptesicus fuscus*. Fig. 26. *Plecotus rafinesquii*. Fig. 27. *Plecotus townsendii*. Fig. 28. *Antrozous pallidus*.

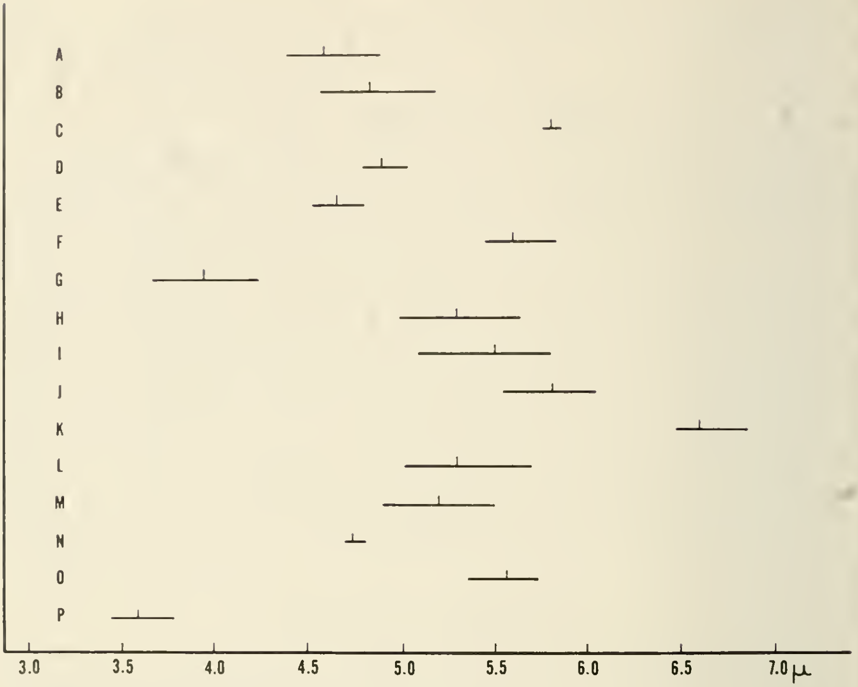


FIG. 29. Comparative head lengths of spermatozoa of some vespertilionid bats. A, *Myotis austroriparius;* B, *M. evotis;* C, *M. grisescens;* D, *M. keenii;* E, *M. nigricans;* F, *M. sodalis;* G, *M. velifer;* H, *M. volans;* I, *Lasionycteris noctivagans;* J, *Pipistrellus subflavus;* K, *Eptesicus fuscus;* L, *Lasiurus borealis;* M, *Nycticeius humeralis;* N, *Plecotus rafinesquii;* O, *P. townsendii;* P, *Antrozous pallidus.* The observed range is indicated by horizontal lines; a vertical line indicates the mean.

dorsal and ventral views; laterally compressed as in most other species of the family Vespertilionidae; length 0.63 (0.51-0.82) based on 10 spermatozoa. *Midpiece* (based on 10 spermatozoa) typical of genus; tapered posteriorly, broadest at anteriormost margin; axial filament prominent; length 15.05 (14.48-15.27), width 1.33 (1.09-1.56).

REMARKS. The spermatozoa of *Myotis evotis* resemble those of *M. volans* in general morphology. Additionally, the head overlaps that of *keenii* and *nigricans* in length. The high degree of variation in length of head in *volans* and *evotis* does not allow separation of the two species on the basis of this character alone; *evotis,* however, has a broader midpiece than does *volans.*

Morphology of spermatozoa within the genus *Myotis* is generally uniform. The head of a typical *Myotis* sperm is short, with nearly parallel sides, blunt apex, slight basal concavity, and is shaped like an isosceles triangle in lateral view (Fig. 2). The neck region is short, but distinct, and laterally compressed. A long, broad midpiece, tapered posteriorly and with a spiral or

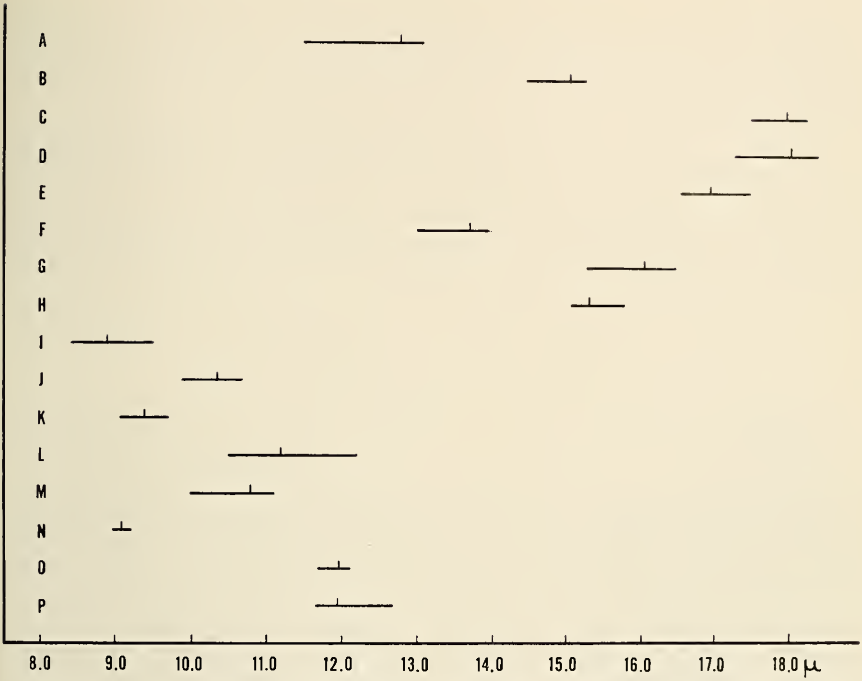


FIG. 30. Comparative midpiece lengths of spermatozoa of some vespertilionid bats. A, *Myotis austroriparius;* B, *M. evotis;* C, *M. grisescens;* D, *M. keenii;* E, *M. nigricans;* F, *M. sodalis;* G, *M. velifer;* H, *M. volans;* I, *Lasionycteris noctivagans;* J, *Pipistrellus subflavus;* K, *Eptesicus fuscus;* L, *Lasiurus borealis;* M, *Nycticeius humeralis;* N, *Plecotus rafinesquii;* O, *P. townsendii;* P, *Antrozous pallidus.* The observed range is indicated by horizontal lines; a vertical line indicates the mean.

helical appearance, normally is seen. The tail is often broad at its point of junction with the midpiece and tapers sharply posteriorly to a narrow filamentous endpiece.

Myotis grisescens A. H. Howell, 1909

Fig. 18

MATERIAL EXAMINED. Two specimens from Kansas.

DESCRIPTION. *Head* (based on 15 spermatozoa) with anterior end dorsoventrally flattened; tapers gradually from posterior to anterior, narrowing to blunt point at apex; posterior margin slightly concave; length 5.81 (5.78-5.87), width 2.53 (2.45-2.63), depth (2 measurements only) 1.59 (1.59). *Neck* well defined; most easily observed in dorsal and ventral views (due to concavity of the base of the head); width uniform; length 0.63 (0.60-0.65) in 15 spermatozoa. *Midpiece* (based on 10 spermatozoa) tapers slightly posteriorly; broad in anterior half; dorsoventrally flattened; distinct axial filament throughout length; heavy granulation often present throughout; length 17.97 (17.49-18.25), width 1.83 (1.71-1.94). *Tail* tapers gradually posteriorly; distinct at point of junction with midpiece; length 48.14 (47.92-48.92) in 6 spermatozoa.

REMARKS. Length of the midpiece exceeds 17.5 in *Myotis grisescens* and *M. keenii*, whereas the midpieces of all other *Myotis* and other vespertilionids studied measure less than 17.5. The head averages longer in *grisescens* than in any other member of the genus studied and, consequently, the spermatozoa of *grisescens* are distinctive among *Myotis*.

Myotis keenii (Merriam, 1895)

Fig. 19

MATERIAL EXAMINED. Two specimens of *Myotis keenii septentrionalis* from Kansas.

DESCRIPTION. *Head* (based on 20 spermatozoa) notably symmetrical with parallel sides, blunt apex, flattened posterior extremity (no concavity); length 4.90 (4.81-5.04), width 2.16 (2.13-2.22), depth 1.29 (1.25-1.34). *Neck* notably shortened in appearance due to lack of concavity in base of head; length 0.53 (0.51-0.60) in 10 spermatozoa. *Midpiece* (measurements based on 10 spermatozoa) extremely long, tapering gently posteriorly; distinct granulation and axial filament observed; typically *Myotis*-like in structure; length 18.02 (17.26-18.39), width 1.41 (1.20-1.48). *Tail* tapers abruptly posteriorly at junction with midpiece, then tapers gradually posteriorly to narrow endpiece; length about 65.0 (3 spermatozoa only).

REMARKS. The spermatozoa of *M. keenii* are remarkably similar to those of *M. grisescens* in length of the midpiece. Spermatozoa of *M. keenii* lack, however, any degree of concavity in the base of the head (a slight concavity is found in *M. grisescens*), and the head is significantly shorter (average 16 per cent) than in *M. grisescens*. Consequently, the spermatozoa of *keenii* are distinct within the genus.

Myotis nigricans (Schinz, 1821)

Fig. 20

MATERIAL EXAMINED. Two specimens of *Myotis nigricans dominicensis* from Dominica (KU 104950, 104953).

DESCRIPTION. *Head* (measurements based on 20 spermatozoa) generally identical in shape to those of other vespertilionids, with rounded apex, slight concavity at base, nearly parallel sides; anterior end dorsoventrally flattened, head "wedge-shaped" in lateral view; relatively short and broad when compared to other *Myotis*, ratio of width to length .48 in *nigricans* compared to .38-.47 in other *Myotis*, length 4.67 (4.53-4.71), width 2.22 (2.17-2.26), depth

1.28 (1.25-1.29). *Neck* evident in dorsal and ventral view, usually distinguishable in lateral view; length 0.68 (0.65-0.74) based on 20 spermatozoa. *Midpiece* (based on 17 spermatoxoa) of moderate length, broader in dorsal or ventral view than in lateral view; tapers gradually posteriorly; length 16.94 (16.54-17.47), width 1.34 (1.25-1.43).

REMARKS. The sperm of *Myotis nigricans* differs from others of the genus in the ratio of length of midpiece to length of head. In *M. nigricans* this ratio averages 3.63; in other *Myotis* it averaged 3.10 or less except in *M. velifer*, in which the average was 4.06. In other words, *nigricans* stands almost midway between the large *velifer* and other species of *Myotis* in this characteristic.

Myotis sodalis Miller and G. M. Allen, 1928

Fig. 21

MATERIAL EXAMINED. One specimen from Missouri.

DESCRIPTION. *Head* (measurements based on 20 spermatozoa) with base slightly concave, apex blunt; length 5.60 (5.45-5.82), width 2.15 (2.03-2.22), depth 1.41 (1.39-1.48). *Neck* well defined and compressed laterally as in the spermatozoa of other species of *Myotis*; length 0.65 (0.65) in 6 specimens. *Midpiece* (based on 3 spermatozoa) appears to taper posteriorly as in spermatozoa of other *Myotis* (although only inadequately stained midpieces observed); an axial filament observed; length averaging 13.7 (recorded as 13.0-14.0 by Hirth, 1960), width 1.40. *Tail* described by Hirth (1960) as tapering gradually to narrow endpiece; length (after Hirth) 65.5 (62.0-69.9).

REMARKS. The spermatozoa of *Myotis sodalis* are distinct among the species of *Myotis* observed on the basis of measurements of the head and midpiece. The ratio of length of midpiece to length of head in *M. sodalis* is 2.45, whereas it is 2.78 (*M. austroriparius*) or larger in other *Myotis* studied. The shortness of the midpiece and unusual length of the head account for the small ratio.

Myotis velifer (J. A. Allen, 1890)

Fig. 22

MATERIAL EXAMINED. Two specimens of *Myotis velifer incautus* from Kansas and one of *M. v. peninsularis* from Baja California (KU 94294).

DESCRIPTION. *Head* (based on 20 spermatozoa) distinctly shorter than in other *Myotis*; base concave, apex blunt, sides nearly parallel in dorsal and ventral views; broad in relation to length; length 3.95 (3.67-4.22), width 1.84 (1.67-2.04), depth 1.32 (1.19-1.50). *Neck* short but distinct; length 0.50 (0.44-0.54) in 10 spermatozoa. *Midpiece* (based on 10 spermatozoa) tapers markedly posteriorly owing to broadened anterior end; axial filament not observed but assumed present; width 92% width of head, more than in any other species of *Myotis*; midpiece 4.06 times longer than head in *velifer*, a greater ratio than in any other vespertilionid (for example, 3.10 in *Myotis evotis*, 2.89 in *M. volans*, 3.09 in *M. grisescens*, 1.60 in *Lasionycteris noctivagans*, and 1.91 in *Plecotus townsendii*); length 16.05 (15.27-16.49), width 1.70 (1.53-1.90).

REMARKS. The spermatozoa of *Myotis velifer* possess characters that allow instant recognition of the species. Most noteworthy is the great length of midpiece in relation to length of head. Also, the length of head is markedly less than that of sperms of other species of *Myotis* studied. These two characteristics allow easy identification of spermatozoa of *velifer*.

Myotis volans (H. Allen, 1866)

Fig. 23

MATERIAL EXAMINED. Three *Myotis volans interior*, one specimen from Arizona (KU 102902), one from North Dakota (KU 100780), and one from South Dakota (KU 100802).

DESCRIPTION. *Head* (based on 20 spermatozoa) typical of *Myotis* in that sides parallel for most of length and apex blunt; base slightly concave; dorsoventral flattening (lateral view) proceeds gradually as in spermatozoa of other *Myotis*; length 5.30 (5.00-5.64), width 2.02 (1.87-2.17), depth 1.37 (1.22-1.50). *Neck* evident as in most other vespertilionids; length 0.59 (0.51-0.65) in 8 specimens. *Midpiece* (based on 5 spermatozoa) narrow in relation to head, narrower than midpieces of other *Myotis* examined; tapers gradually posteriorly; axial filament centrally located; length 15.30 (15.06-15.78), width 1.12 (1.05-1.26).

REMARKS. The spermatozoa of *Myotis volans* are generally characteristic of the genus of *Myotis*. One character, breadth of the midpiece, distinguishes *volans* from the other species examined. *M. volans* averages 1.12 (1.05-1.26), which is 16 per cent less than breadth of midpiece in *evotis*, and 39 per cent less than in *grisescens*. The narrowness of the midpiece in *M. volans* is diagnostic. Also, the head is somewhat narrower in *volans* than in other vespertilionids, but extensive variation in this character suggests that its significance is questionable.

Lasionycteris noctivagans (LeConte, 1831)

MATERIAL EXAMINED. None.

DESCRIPTION. *Head* nearly symmetrical with rounded but somewhat narrowed apex, according to Hirth (1960:78); widest in basal half; length 5.5 (5.1-5.8), width 2.0 (1.9-2.1). *Neck* well defined, broadest anteriorly. *Midpiece* tapered posteriorly; no apparent helical configuration; length 8.9 (8.4-

9.5), width 0.8 (0.7-1.0). *Tail* relatively long; tapered posteriorly; length 73.4 (65.1-79.2).

REMARKS. Hirth's *(loc. cit.)* data indicate that spermatozoa of *L. noctivagans* are similar to those of other vespertilionids.

Pipistrellus subflavus (F. Cuvier, 1832)

Fig. 24

MATERIAL EXAMINED. Three specimens of *Pipistrellus subflavus subflavus* from Kansas.

DESCRIPTION. *Head* (based on 10 spermatozoa) ovate, slightly broader anteriorly than posteriorly; marked basal concavity; apex slightly more pointed than in *Myotis;* sides never parallel; length 5.81 (5.54-6.05), width 2.50 (2.35-2.65), depth 1.48 (1.48). *Neck* short, distinct; length 0.61 (0.61) in 5 spermatozoa. *Midpiece* (based on 10 spermatozoa) short and narrow; posterior limit often indistinct; midpiece broadest at anterior extremity, tapers sharply posteriorly; anterior swelling reported by Hirth (1960:78) not observed by me; length 10.35 (9.87-10.67), width 0.82 (0.82). *Tail* tapers gradually from broad anterior part to narrow endpiece; length about 70 in 2 spermatozoa.

REMARKS. The spermatozoa of *Pipistrellus subflavus* are similar to those of *Eptesicus fuscus* in shape of the head and the midpiece. However, the midpiece of *P. subflavus* is distinctly longer and the head shorter than in *E. fuscus.* The shape of the head in combination with length of the midpiece readily distinguish spermatozoa of *P. subflavus* from those of other vespertilionids, including *E. fuscus.*

Eptesicus fuscus (Palisot de Beauvois, 1796)

Fig. 25

MATERIAL EXAMINED. One specimen of *Eptesicus fuscus pallidus* from Arizona (KU 102096).

DESCRIPTION. *Head* (based on 20 spermatozoa) extremely large, oval, slightly concave basally (Hirth, 1960, described head as "narrowly obovate" but description inconsistent with my observations); sides of head not parallel, tapering slightly posteriorly and anteriorly—broadened anterior region described by Hirth not observed; unusual length possibly due to presence of stainable acrosomal cap (but most likely acrosome not observed, the lightly-colored anterior portion of head resulting from dispersed nuclear material—head measurements include this light anterior portion); length 6.59 (6.47-6.85), width 2.71 (2.59-2.77), depth 1.31 (1.28-1.33). *Neck* short; centriole ring observed under phase microscopy as described by Fawcett and Ito (1965) for spermatozoa of *Myotis lucifugus*; length 0.50 (0.46-0.55) in 8 specimens.

Midpiece (based on 4 spermatozoa) extremely short in relation to head—distinct within family on basis of this character; ratio of length of midpiece to length of head 1.42 in *E. fuscus*, 1.62 in *Lasionycteris noctivagans* (Hirth, 1960:78), and more than 1.90 in all other vespertilionids studied; narrow, broadest at anterior extremity, tapering sharply posteriorly; spiral, helical nature observed throughout; length 9.39 (9.06-9.70), width 0.92 (0.92). *Tail* not observed as complete in my study; Hirth *(loc. cit.)* recorded length of tail as 72.0 (68.1-75.1).

REMARKS. Using sperm morphology, *Eptesicus fuscus* may be distinguished from other vespertilionids on the basis of length of head alone and also length of head in relation to length of midpiece. Length of head in *E. fuscus* exceeds 6.47, whereas other vespertilionid spermatozoa do not exceed 6.10. Ratio of length of head to length of midpiece is .70 in *E. fuscus,* and less than .55 in all species studied excepting *Pipistrellus subflavus* (.56), and *Lasionycteris noctivagans* (.62). Differences in gross morphology between the spermatozoa of *Eptesicus* and *Lasionycteris* make them readily distinguishable (see Hirth, *loc. cit.*).

It is noteworthy that no mature spermatozoa were observed in adult *E. f. pallidus* and *E. f. fuscus* collected in Kansas in November and December, although immature spermatozoa were found in abundance in many of these bats. Mature spermatozoa have been reported in testes and male ducts of *E. f. fuscus* in late autumn (Guthrie, 1933).

Lasiurus borealis (Müller, 1776)

MATERIAL EXAMINED. None.

DESCRIPTION (after Hirth, 1960:79). *Head* with concave base; apex blunt and broadly rounded; length 5.3 (5.0-5.7), width 2.0 (1.9-2.3). *Midpiece* not spiraled (or if so, not reported); evident swelling near posterior end; length 11.2 (10.5-12.2), width 0.8 (0.7-1.0). *Tail* nearly uniform in width throughout; length 67.0 (64.0-70.0).

REMARKS. Hirth mentioned no unique features of the spermatozoa of this bat. Compared with the spermatozoa of vespertilionids I have studied, however, those of *L. borealis* seem unique (based on Hirth's figure) in having a bulge in the posterior region of the midpiece.

Nycticeius humeralis (Rafinesque, 1818)

MATERIAL EXAMINED. None.

DESCRIPTION (after Hirth, 1960:79). *Head* elliptical; base somewhat truncate; no basal concavity; length 5.2 (4.9-5.5), width 2.1 (2.0-2.4). *Neck* short, distinct. *Midpiece* and *tail* exceptionally narrow, midpiece with slight helical appearance; both midpiece and tail tapering gradually throughout;

length of midpiece 10.8 (10.0-11.1), width of midpiece 0.9 (0.8-1.1); length of tail 77.3 (72.0-84.1).

REMARKS. The spermatozoa of *Nycticeius humeralis* appear to be distinct within the family Vespertilionidae in that the head is elliptical-shaped and lacks a flattened or concave base (Hirth, 1960:79).

Plecotus rafinesquii Lesson, 1827

Fig. 26

MATERIAL EXAMINED. One specimen of *Plecotus rafinesquii rafinesquii* from Kentucky.

DESCRIPTION. *Head* (based on 20 spermatozoa) tapers gradually anteriorly (lateral view, narrowing to blunt point at apex; dorsoventrally flattened at anterior end); posterior limit with minute concavity; lateral extremities nearly parallel in posterior two-thirds of head; length 4.74 (4.71-4.81), width 2.25 (2.17-2.36), depth 1.53 (1.52-1.57). *Neck* distinct in lateral as well as dorsal and ventral views (base of head does not hide neck region); width uniform; length 0.53 (0.46-0.55) in 12 spermatozoa. *Midpiece* (based on 10 spermatozoa) tapers gradually posteriorly; axial filament distinct; regular patches of granulation suggest helical configuration as reported by Hirth (1960:79); constricted slightly at point of junction with tail; dorsoventral flattening little marked; length 9.06 (8.96-9.20), width 1.41 (1.39-1.48). *Tail* tapers only slightly posteriorly; length of tail approximately 45 in my material although Hirth *(loc. cit.)* reported 50.0 (46.1-57.3).

REMARKS. *Plecotus rafinesquii* can be distinguished readily from *P. townsendii* using morphology of spermatozoa. The ratio of length of head to length of midpiece is approximately the same (.52) in the two species, but the head of *townsendii* exceeds 5.2 in length, whereas that of *rafinesquii* is less than 5.2, and length of midpiece is greater than 11.0 in *rafinesquii* (Fig. 30). Also the head is more spade-shaped in *townsendii* than in *rafinesquii* (Figs. 26, 27).

The sperms of *P. rafinesquii* are typical of the family Vespertilionidae in that they have the characteristic shape of the head (apex blunt, sides nearly parallel), have a distinct neck, and have a midpiece that tapers posteriorly. The spermatozoa of *rafinesquii* differ from those of several other vespertilionids in having a shorter tail (50.0 or less in most specimens as opposed to more than 60.0 in some other species studied).

It is noteworthy that the one specimen of *P. rafinesquii* examined, collected in October, has vas deferentia that contained unusually large quantities of mature spermatozoa, whereas several specimens of *P. townsendii* collected in November in Kansas were virtually devoid of mature sperms. One specimen of *townsendii* collected in South Dakota in June also was devoid of mature spermatozoa.

Plecotus townsendii Cooper, 1837

Fig. 27

MATERIAL EXAMINED. Two specimens of *Plecotus townsendii pallescens* from Kansas.

DESCRIPTION. *Head* (based on 20 spermatozoa) concave at posterior limit; apex not so blunt as in other vespertilionids; sides (in dorsal or ventral view) tapering slightly anteriorly; head similar to that of *P. rafinesquii,* but somewhat more spade-shaped and dimensions greater; length 5.56 (5.36-5.73), width 2.70 (2.54-2.87), depth 1.67 (1.57-1.80). *Neck* short, discernible in dorsal and ventral views only; evidently uniform in width; length 0.63 (0.60-0.67) in 6 spermatozoa. *Midpiece* (based on 10 spermatozoa) broad, of moderate length (shorter than in *Myotis,* but longer than in *Eptesicus fuscus*); coarsely granulated and slightly tapered posteriorly; abruptly tapered at junction with tail; distinct, deeply-staining axial filament observed; length 11.97 (11.69-12.11), width 1.52 (1.48-1.59).

REMARKS. *P. townsendii* is distinct from all other vespertilionids studied, except *Antrozous pallidus* and *Myotis austroriparius,* on the basis of length of midpiece. Length of head (average 5.56) is considerably greater in *P. townsendii* than in *A. pallidus* (average 3.79) and breadth of midpiece distinguishes *townsendii* from *M. austroriparius*; hence, the spermatozoa of *townsendii* are distinct from all others studied. The two species of *Plecotus* studied have similar spermatozoa but those of *townsendii* are larger in several measurements as explained in the account of *rafinesquii.*

Subfamily **Nyctophilinae**

Antrozous pallidus (LeConte, 1856)

Fig. 28

MATERIAL EXAMINED. Two specimens of *Antrozous pallidus bunkeri* from Oklahoma.

DESCRIPTION. *Head* (based on 20 spermatozoa) with apex extremely blunt (dorsal and ventral view), sides nearly parallel (tapered slightly posteriorly); degree of concavity in base less than in other vespertilionid spermatozoa and concavity not evident in all preparations; head, in lateral view, with shape of isosceles triangle (dorsoventrally flattened anteriorly); head appears deep owing to stubbiness; length 3.59 (3.42-3.79), width 2.54 (2.40-2.59), depth 1.86 (1.71-2.01). *Neck* region between posterior limit of head and anterior margin of midpiece poorly resolved in my preparations; neck observed in some spermatozoa but poorly defined and minute. *Midpiece* (based on 10 spermatozoa) granular in appearance, spiraled regularly throughout most of length; tapers slightly posteriorly; axial filament observed; broad in relation to head, breadth nearly uniform throughout (slight posterior tapering); marked difference in dimensions of posterior end of the midpiece and anterior part of tail (unique among vespertilionids studied); length 11.95 (11.65-12.66), width 2.15 (2.03-2.26). Occasional spermatozoa (probably immature) were observed with large clumps of cytoplasm clinging to the anterior one-third of the midpiece. These cytoplasmic aggregations probably remained from the process of cell maturation as described by Bloom and Nicander (1961). *Tail* uniquely bulged just posterior to junction with midpiece, tapered gradually to distal end; length approximately 43.

REMARKS. The sperm of *Antrozous pallidus* is unique among that of vespertilionids of this study in several characters. One distinctive feature is the short, broad head. The ratio of breadth of head to length of head in *A. pallidus* is .71, whereas other species of vespertilionids yield a ratio of less than .50. Other features include the distinctly broader midpiece that terminates abruptly with little tapering posteriorly, and a prominent anterior bulge in the tail.

The genus *Antrozous* is presently separated taxonomically from other North American vespertilionids by assignment to the subfamily Nyctophilinae, first suggested by Peters in 1865 and later supported by Miller (1907). The distinct spermatozoa of *Antrozous* support this arrangement.

DISCUSSION

PHYLLOSTOMATIDAE

Although spermatozoa of species representing only five of the seven recognized subfamilies of the Phyllostomatidae were examined, it is immediately apparent that greater uniformity in sperm structure prevails in this family than in the family Vespertilionidae. This is particularly demonstrated in configuration and dimensions of the midpiece and shape of the head.

At the subfamilial level, only the Glossophaginae and the Sturnirinae (two species of each subfamily studied) appear unique. Extensive overlap in dimensions of sperms and similarity in gross morphology suggest close taxonomic affinities among the various taxa within the family. Here, as in the Vespertilionidae, there appears to be no correlation between size of the bat and size of the sperm.

Glossophaga soricina and *Anoura culturata,* the two glossophagines studied, are separable from other phyllostomatids in having a short midpiece and clearly should be grouped together. Only the presence of a distinct neck region distinguishes the spermatozoa of *Carollia castanea* (Carolliinae). Necks may be found in other phyllostomatid spermatozoa, and I am therefore reluctant to regard the presence of a neck in *C. castanea* as distinctive for the subfamily. The spermatozoa of *C. castanea* are otherwise similar to those of *Artibeus lituratus* and to two species of *Sturnira,* particularly in general size and configuration of head and midpiece.

The sperm of *Sturnira lilium* is consistently smaller than that of *S. ludovici* (Figs. 11, 12) in both length of head and length of midpiece. The spermatozoa of Sturnirinae appear to be unique among phyllostomatids studied in lacking a concavity at the base of the head.

Two species of *Artibeus* (subfamily Stenoderminae) were examined. The spermatozoa of *Artibeus jamaicensis* have heads and midpieces that are consistently shorter than those of *Artibeus lituratus,* but the two species do not differ markedly from other members of the family on the basis of overall sperm morphology.

Because the sperms of only a few phyllostomatids were examined, it is not possible to discuss relations among the subfamilies. It is clear, however, that variation in sperm structure is slight among genera of different subfamilies, and, on the basis of sperm morphology, the genera and species studied seem more closely allied than do comparable taxa within the family Vespertilionidae. Other morphological characters presently distinguish groups of phyllostomatid bats at the subfamilial level. Available information regarding sperm morphology suggests that a re-evaluation of subfamilial categories may be warranted.

VESPERTILIONIDAE

In some instances, morphology of spermatozoa reinforces presently recognized taxonomic categories within the family Vespertilionidae; in other instances, questions regarding the validity of some taxa are raised. The following discussion is not intended as the basis for taxonomic changes, but simply points out some current taxonomic arrangements that possibly are in need of re-evaluation.

Within the Vespertilionidae, variation in length of the sperm head is 3.59 in *Antrozous pallidus* to 6.85 in *Eptesicus fuscus.* Considerable variation was found also in length of the midpiece, 9.06 in *E. fuscus* to 18.25 in *Myotis grisescens.* In general, it may be said that variation at the generic and specific levels is much greater in vespertilionid spermatozoa than in those of phyllostomatids, both in measurable dimensions and gross morphology. In fact, variation is greater between genera in this family than between subfamilies of phyllostomatids. In at least one genus, *Myotis,* interspecific variation is marked.

The species of vespertilionids studied may be separated into two groups on the basis of length of the midpiece (Fig. 30). Species studied of the genus *Myotis* all had midpieces greater than 12.75 μ in average length, whereas all others observed were less than 12.75 (including *Pipistrellus, Eptesicus, Nycticeius, Lasiurus, Plecotus,* and *Antrozous*).

No obvious subgroups were evident within *Myotis.* Although variation was found to be extensive, most species are recognizable using some character (or combinations of characters) of the spermatozoa. *Myotis evotis* was the only species examined in which the sperm does not appear unique in at least one characteristic; the spermatozoa of *evotis* overlap those of *volans* and *velifer* in length of the midpiece and those of several species in length of the head. The spermatozoa of *volans* are remarkably similar to those of *evotis* in lengths of head and midpiece but are distinguished from *evotis* in having a narrower midpiece. *M. grisescens* and *M. keenii* appear to be distinct owing to their unusually long midpiece. These two species are easily separated from one another because the spermatozoa of *keenii* have much shorter heads than those of *grisescens.* In *M. nigricans,* the long midpiece and short head yield a unique ratio among the *Myotis* studied. The spermatozoa of *M. sodalis* are distinct in having the shortest midpiece of any kind studied (excepting *M. austroriparius*—Hirth, 1960); additionally, *sodalis* spermatozoa have long heads, resulting in a unique ratio between length of midpiece and length of head. The spermatozoa of *M. velifer* are distinct in having exceptionally short heads, nearly .3 microns shorter than those of any other *Myotis* observed; however, *M. austroriparius* also has been described as having a short head (Hirth, 1960), but this species has an extremely short midpiece as well.

The spermatozoa of *Lasionycteris* appear to have characters in common with those of *Pipistrellus.* The two are similar in general structure of the midpiece, and Hirth (1960) reported a round, symmetrical head with a narrowly rounded apex in *Lasionycteris* that is similar to what I observed in the spermatozoa of *Pipistrellus subflavus.* Hirth's description of head structure in *Pipistrellus* differs from my own, suggesting that more work is needed to determine variation before truly accurate comparisons can be made.

Although similar to *Eptesicus fuscus,* the spermatozoa of *Pipistrellus subflavus* are distinct (head somewhat shorter than *E. fuscus*) within the family. The sperms of both *Pipistrellus* and *Eptesicus* have large, ovate heads and short, narrow midpieces. In past taxonomic considerations of these two genera, some authors have noted the similarities in cranial morphology between *Eptesicus* and especially Old World species of *Pipistrellus* (Miller, 1907; Tate, 1942). My data suggest close taxonomic affinities between these two genera. It is of interest to note, for example, that interspecific variation in sperm morphology is greater among species of *Myotis* than between *Eptesicus fuscus* and *Pipistrellus subflavus.*

The spermatozoa of *Nycticeius humeralis* and *Lasiurus borealis,* as illustrated by Hirth (1960), have features that readily distinguish them from those of other vespertilionids. The spermatozoa of *Nycticeius* have a broadly elliptical head, and those of *Lasiurus* have a prominent bulge in the posterior region of the midpiece. These characters are unique to the two genera and set each well apart from other genera of Vespertilionidae.

The spermatozoa of *Plecotus* clearly resemble those of *Myotis* more than they resemble spermatozoa of any other vespertilionids examined. Both *Plecotus* and *Myotis* have heads with nearly parallel sides, concave bases, and blunt apexes, and the midpieces of both are broad and taper gradually posteriorly. Although a number of conventional morphological characters distinguish species of these two genera (structure of the skull, number of cheekteeth, size of auditory bullae, and length of ear, for example), spermatozoan morphology suggests that *Myotis* is more closely related to *Plecotus* than to any other genus studied.

The spermatozoa of *Plecotus townsendii* and *P. rafinesquii* can be distinguished from one another on the basis of absolute dimensions; the head and midpiece are, however, similar in proportion. Ratios of length of head to length of midpiece, and width of head to length of head are essentially equal in the two species. These facts, along with similar data for two species of *Sturnira* (Phyllostomatidae), suggest that increase in size of the sperm head with a corresponding increase in midpiece length may be an expression of the maintenance of the nucleoplasmic index (increase in nuclear mass with an increase in the cytoplasmic matrix) within these genera. There are, however, exceptions to this trend (*e.g., Myotis velifer* and *M. nigricans*) that do not allow universal application of the principle.

Studies of spermatozoa from *Antrozous pallidus* indicate that this bat is clearly distinct at a supergeneric level among vespertilionids examined. A short, thick head, greatly enlarged midpiece, and anterior bulge in the tail are unique and placement of *A. pallidus* in a different subfamily, Nyctophilinae, from other American species seems justified.

There appears to be no direct correlation between the size of a bat and size of spermatozoa in vespertilionids. For example, the spermatozoa of the large *Myotis grisescens* and *M. velifer* are large, but those of the small *M. nigricans* and the medium sized *M. keenii* also are large, and spermatozoa of *Plectous rafinesquii* are comparatively small.

SUMMARY

Spermatozoa from 16 species of vespertilionid and eight species of phyllostomatid bats were studied, and compared morphologically with reference to the taxonomy of the two groups. Among the vespertilionids examined variation at the generic level was greater than at the subfamilial level in phyllostomatids. For example, the genus *Myotis* was found to be separable from all other vespertilionid genera studied in length of the midpiece alone, demonstrating uniformity in this character within the genus.

Using sperm morphology as a criterion, relationships of bats studied generally were in agreement with the systematic arrangements presented by Hall and Kelson (1959). All genera and most species of vespertilionids were found to be distinct in morphology of spermatozoa, whereas only a few genera and species of phyllostomatids were readily separable from other members of the family. Spermatozoa examined from bats of two different subfamilies of vespertilionids are highly distinctive.

Although large bats normally have relatively large spermatozoa, no direct size relationship was evident. Some evidence was found to suggest an increase in length of the midpiece with an increase in size of the head, but exceptions to this trend were noted.

This investigation has demonstrated the potential usefulness of spermatozoan morphology in taxonomic and evolutionary studies of bats and suggests the need for additional, more detailed, work in this area.

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A Revision of the Genus *Brachygastra* (Hymenoptera: Vespidae)[1]

Martin G. Naumann

ABSTRACT

This paper is a taxonomic revision of the genus *Brachygastra*, which occurs in Central and South America. Twelve species are recognized. *Brachygastra fistulosa* is new, and *B. smithii, B. propodealis, B. mellifica* and *B. borellii* are elevated to specific rank. Eleven varieties are placed in synonymy and their status in taxonomy is discussed. All species are described and the available knowledge of their biology and distribution is summarized.

INTRODUCTION

Recent treatments (Bequaert 1933, 1943, 1944) of the genus *Brachygastra* (= *Nectarina*) have concerned only a few species or certain geographical regions. These studies, while contributing much to the knowledge of the genus, have confounded rather than clarified the status of many of the described forms. Approximately 25 forms have been assigned to the genus. Many of these were described as color forms or varieties and some others, although originally described as distinct species, have been considered varieties. The present study is a taxonomic revision of the genus *Brachygastra* and a reevaluation of the various forms in the light of recently acquired material.

The genus *Brachygastra* is one of the common elements of the neotropical fauna. It extends from the southwestern United States (southern Arizona and Texas) to Argentina (Buenos Aires). The species build phragmocyttarous nests which in species of the *lecheguana* group are perennial and may become very large with populations of as many as 15,000 wasps. In other species the nest does not attain great size and does not appear to last as long. The species are pleometrotic, the nests being founded by swarming, and the

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queens comprising as much as 17% of the population (Buysson, 1905, for *B. lecheguana*).

As the name indicates, the wasps of this genus are best recognized by the short, truncate abdomen which is usually wider than long in preserved specimens. In addition, the scutellum is very high and often projects over the metanotum. The genus closely resembles *Chartergus* and *Pseudochartergus*, to which it is closely allied.

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MATERIALS AND METHODS

Approximately 4,700 pinned specimens have been examined during the course of this study. These are distributed among the species as follows:

	♀♀	♂♂
Brachygastra augusti (Saussure)	467	4
B. azteca (Saussure)	1,031	7
B. baccalaurea (R. von Ihering)	22	0
B. bilineolata Spinola	324	1
B. borellii (Zavattari)	10	..*
B. buyssoni (Ducke)	3	..*
B. fistulosa n. sp.	9	..*
B. lecheguana (Latreille)	890	27
B. mellifica (Say)	1,386	58
B. propodealis Bequaert	8	..*
B. scutellaris (Fabricius)	403	0
B. smithii (Saussure)	102	2

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* Males not known.

A large number of specimens is not necessarily indicative of wide distribution of specimens throughout the range of a species since large nest series are often involved.

All measurements were made with the aid of an adjustable eyepiece micrometer on a binocular microscope, calibrated with a stage micrometer. All drawings were made with the aid of an ocular grid.

Descriptions of species in which there is a great deal of geographical variation refer to the condition of the species at the type locality and variations throughout the range are described in a separate section. Characters are numbered to facilitate comparison among descriptions. Ratios and measurements given are means ± 1 standard error. Descriptions of coloration are given for the forms in which maculations are most developed and the variations are discussed following the description.

Lists of localities are divided into countries and each country into the appropriate subdivisions (Estado, Departamento, etc.). Parentheses indicate that the given locality could not be located on any map or gazeteer examined. Brackets include additional information supplied by me.

Synonymy is given in abbreviated form. A complete listing is available in the thesis in The University of Kansas library.

TERMINOLOGY

Unless otherwise indicated, terminology is that of Duncan (1939).

Size. Although total body length is occasionally indicated, it is no more than a rough approximation as the position of the abdomen varies greatly in preserved specimens. Where indicated, body length is the approximate length of head, thorax and the first two gastral segments in horizontal position (h + th + terg 2). A more reliable indication of size is the length of the forewing which is measured from the apex of the humeral plate to the apex of the wing.

Punctures and pubescence. As punctures are often diagnostic in the genus, it is important that they are properly understood. Size is indicated relative to width of the median ocellus, *i.e.* small are ¼ or less than, medium are ¼ to ½, and large are ½ or greater than the width of the ocellus. Density is indicated by average number of diameters of a single puncture between punctures. Deep punctures are those which are about as deep as wide. Texture of the surface, *i.e.* smooth or rugulose, refers to the surface of the cuticle between the punctures. Length of hairs is likewise indicated in relation to ocellar width.

Head dimensions. Height is the distance from the apex of the clypeus to the top of the ocular swelling in frontal view (h, Fig. 1). Length is the approximate distance from the occipital carina to front of the vertex in dorsal view (l, Fig. 5). Width is the maximum width of the head including the eyes (w, Fig. 1).

Ocelli. Distances between the lateral ocellus and the eye (eo, Fig. 5) and the occipital carina (co) are given in proportion to the distance between the inner margins of the lateral ocelli (oo).

Gena. Width of the gena is the distance between the margin of the eye and the occipital carina at the various points indicated. The postgenal convexity is the convexity of the lower third of the postgena, seen on the gena as a more or less developed convexity of the ventral portion of the posterior margin (pg c, Fig. 47).

Clypeus. Width of the clypeus is measured between the mesal margins of the mandibular condyles (cw, Fig. 1). Length is the distance between the apex and the median point of the epistomal suture. The contact with the eye is the distance from the point of contact of the epistomal suture to the ventral curvature of the eye (c, Fig. 1). The lateral clypeal lobes are the lateral extensions of the distal margin of the clypeus and the apical triangle is the portion of the clypeus set off by a line drawn between the ventral margins of these lobes (ll, tr, Fig. 1).

Malar space. The malar space is the vertical length of the subgena immediately posterior to the first mandibular condyle (m, Fig. 2).

Pronotum. Because of the angular nature of the pronotum, it is necessary to designate a dorsal surface as distinguished from anterior and lateral surfaces. These three surfaces are more or less distinct from each other depending on the development of the pronotal keel and the humeral angle. The humeral angle is the shoulderlike development of the anterolateral surface of the pronotum. (h, Fig. 9), and it may bear the enlarged and anteriorly inflected pronotal keel (k, Fig. 44). In genera such as *Polybia* and *Synoeca* the humeral angle may be entirely absent, the pronotum forming an almost flat oblique surface.

Scutellum. Like the pronotum, the scutellum is more or less angular and may have a distinct horizontal, dorsal surface and a vertical, posterior surface. The lateral surfaces, if likewise distinct, form the variable scutellar pockets (scu p, Fig. 15). If the margins between these surfaces are sharp, the scutellum is said to be angular. If the margins are rounded, the scutellum is termed rounded. The scutellum is bilobed when there is a median, posterior emargination (Fig. 12). The length and width of the respective surfaces is the median measurement unless otherwise indicated (l, w, Fig. 9).

Propodeum. The propodeum is entirely vertical and is divided into posterior and lateral surfaces, corresponding to the dorsal and lateral surfaces of the extended propodeum of the *Polybia*-like genera. The propodeal angle is the lateral development of the propodeum (pr a, Fig. 13) and is not to be confused with a compressed lobe (pr l) which is often present on the propodeal angle which is actually an extreme development of the lateral ridge (lr).

Abdomen. The term abdomen is here used to designate the gaster or metasoma. Metasomal segments are numbered with Arabic numerals whereas the true abdominal segments are designated by Roman numerals (*i.e.,* tergum 2 = tergum III). Arabic numerals are used throughout the species treatments. The length and width of a tergum are the maximum median measurements.

Genitalia. Terminology here follows that of Snodgrass (1941). The volsella consists of a large digitus (= tenette of Buysson) which articulates with the volsellar plate, and a small cuspis (= volsella of Buysson) which is appressed against the mesal margin of the paramere (cu, Fig. 17). The digitus bears a distal lobe of variable shape (dl, Fig. 17). The posterior angle of the digital lobe is the form of the apex of the lobe, and the ventral angle is the form of the ventral process of the lobe (pa, va, Fig. 18). The volsellar lobe (= appendice of Buysson) is here used to designate the fingerlike lobe arising proximal to the digitus (vl, Fig. 17), which seems to be unique among the Polybiinae. Araujo (1946) has figured *Protonectarina* as having a small volsellar lobe but to my knowledge no other polybiine has this structure.

TAXONOMIC CHARACTERS

It is unfortunate that in taxonomy the most obvious and attractive characters are often emphasized and receive unjustified weight over other, more subtle characters, whether or not they merit such treatment. Such has often been the case in the taxonomy of *Brachygastra* and of the social Vespidae in general. Color, because of its predominant effect on the appearance of the wasp, has received considerable attention. Many species have been described on the basis of color alone and numerous "varieties" or "color forms" have been designated without indication whether the form was a geographical variant, a local color form or a subspecies. Consequently the treatment of the numerous forms varies, resulting in taxonomic and nomenclatorial confusion. I am by no means condemning the use of color as a character in the Vespidae, but rather the emphasis on color characters without prior consideration of the stability of the character both within populations and throughout the ranges of species.

It has been shown (Enteman, 1904) that color pattern in *Polistes* may be correlated, at least in part, with environmental conditions. Richards and Richards (1951) found the extent of the yellow color pattern in *Brachygastra scutellaris* to be correlated with ovarial development. At least one described form, *Nectarinia rufiventris* Saussure, may be based on a teneral specimen. The occurrence of yellow forms in both *Brachygastra scutellaris* and *B.*

bilineolata in the dry savannas of northern South America, and the predominance of black pigmentation in other species of *Brachygastra* found in high altitudes also suggests that environment has an effect on pigmentation, although there are doubtless genetic components involved.

I question the value of naming varieties when these are merely variations within a population. For the most part the long lists of varieties in species such as *Polistes canadensis* (Linnaeus) and *Brachygastra scutellaris* (Fabricius) are unnecessary and cumbersome. Some of the varieties may be distinct species, as has been found in both *Polistes* and *Brachygastra,* but a large number are probably only color forms and hence need not and should not be given names.

The present classification is based on more stable characters such as the occipital carina, pronotum, scutellum, metanotum and propodeum. The cuticular structures such as punctures and hairs are also used but color pattern is considered only on a qualitative basis.

The best characters are found in the male genitalia but unfortunately the males are seldom collected and in some species are entirely unknown. It is on the basis of male characters that *B. mellifica* (Say) and *B. smithii* (Saussure) have been recognized in this study. Variation among the females of some species indicates the possibility of their separation into additional species, but too little is known to justify such separation at present.

This study of the genus, then, cannot be considered a complete and satisfactory treatment, but rather a reevaluation on the basis of the material available.

Brachygastra Perty

Brachygastra Perty, 1833, Delectus Anim. Articul. Brasil, p. 145. (Type species, *Brachygastra analis* Perty = *Polistes lecheguana* Latreille; designated by Bequaert, 1932).

Nectarina Swainson and Shuckard, 1840, On the History and Natural Arrangement of Insects, p. 183, foot note. (Type species, *Brachygastra analis* Perty = *Polistes lecheguana* Latreille; designated by Ashmead, 1902) (New name for *Brachygastra* Perty, 1833).

Melissaia Shuckard, 1841, *in* White, Ann. Mag. Nat. Hist. 8:320, foot note (new name for *Nectarina* Swainson and Shuckard, 1840).

Brachygaster Saussure, 1852, Et. Fam. Vesp. 1:171, foot note (misspelling of *Brachygastra* Perty, 1833). Not *Brachygaster* Leach, 1817 (Hym.).

Nectarinia Saussure, 1853-1858, Et. Fam. Vesp. 2:225 (misspelling of *Nectarina* Swainson and Shuckard, 1840). Not *Nectarinia* Illiger, 1811 (Aves).

Caba R. von Ihering, 1904, Rev. Mus. Paulista 6:105. (New name for *Nectarinia* Saussure, 1853-1858).

The genus *Brachygastra,* as first proposed by Perty in 1833, included two species, *B. analis* Perty and *B. scutellaris* Perty. Perty's name, *Brachygastra,* was subsequently replaced with *Nectarina* by Swainson and Shuckard who considered it a homonym of *Brachygaster* Leach, 1817. In proposing the new name they inadvertently created a homonym of *Nectarinia* Illiger, 1811, a genus of Sunbirds (Nectariniidae). Consequently Shuckard the following year changed the name to *Melissaia.* Saussure in his monograph used the name *Nectarinia,* and included ten species in the genus. At the same time

Smith (1857) used, once again, the name *Nectarina* and both names were used until Bequaert (1932) recognized *Brachygastra.* R. von Ihering had attempted to solve the nomenclatorial problem by proposing the name *Caba* to replace *Nectarinia* but his name was not widely used due to the general acceptance of *Nectarina.* As late as 1932, *Nectarina* was used in preference to *Brachygastra* even though the validity of the latter name was recognized.

Diagnosis. *Brachygastra* is easily recognized by the prominent, projecting scutellum which, together with the metanotum and propodeum, forms the flat, vertical posterior surface of the thorax (Fig. 13). The first abdominal segment is short, cap-shaped, and not at all petiolate. The second segment is greatly enlarged, and often conceals the succeeding segments, giving the wasps their characteristic, short form (Fig. 68). Several polybiine genera such as *Pseudochartergus, Chartergus* and *Parachartergus* are quite similar to *Brachygastra* in general appearance but differ on the basis of the above characters as well as the more obvious generic characters such as mouthparts.

Female. Length (h + th + terg 2) 5-9 mm. Wing length 5-9 mm.

Head (Figs. 1, 2, 5). In frontal view little broader than high, rounded, only slightly narrower ventrally than dorsally; in dorsal view 2.5-3 times as wide as long, posterior margin more or less curved. Ocelli normal. Vertex more or less convex, with median ocular convexity more or less developed. Frons slightly convex, interantennal convexity variable, slight. Clypeus 1.4 to 1.8 times as wide as long, more or less convex, distal margin variable, broadly rounded to pointed; epistomal suture evenly curved or V-shaped dorsally. Anterior tentorial pit deep; immediately below antennal socket. Subantennal suture not visible. Malar space very small to about .8 width of antennal socket. Eye moderately emarginate, more or less pubescent. Gena variable, .7 to 1.4 times as wide as eye in lateral view, often with large postgenal convexity, more or less distinct from postgena. Occipital carina variable, very low, incomplete (Fig. 2), to very high, complete (Fig. 47); never absent. Postgena with large lateral sulcus. Labrum small, retracted, concealed under clypeus, tab-like, lightly sclerotized. Maxilla (Fig. 8) with cardo and stipes sclerotized; lacinia lobe-like, almost entirely membranous, with only elongate median area lightly sclerotized; galea sclerotized, subdivided distally; palpus normal, 6-segmented. Labium (Fig. 6) with prementum strongly convex, heavily sclerotized, with long narrow median emargination distally; anterior and posterior lingual plates membranous or very lightly sclerotized; glossae broad, rounded, fused in basal two thirds; paraglossae narrow; acroglossal buttons present, well sclerotized; palpus 4-segmented. Mandible (Fig. 1) with 5 teeth, apical 3 large, acute, basal 2 low, rounded; with two blunt teeth on posterior surface. Antenna with scape about .5 as long as flagellum, slightly curved; pedicel about as long as wide, swollen; flagellum more or less swollen, basal flagellomere about as long as succeeding two flagellomeres.

Thorax (Figs. 9, 13, 15). Cuboid, about as long as high; posterior surface flat, nearly parallel with anterior surface. Pronotum variable, rounded to angular; humeral angle absent to well developed; pronotal keel variable. Scutum wider than long. Scutellum strongly convex, prominent, rounded to angular, often extending over plane of metanotum; axilla large, vertical, formed into a ridge dorsally; scutellar crest forming an elongate, flattened, blade-like flange. Metanotum flat; dorsal and ventral margins more or less parallel; metanotal depression large, deep. Mesopleuron strongly convex, entire, not subdivided by any suture. Metapleuron divided into a small dorsal sclerite and a larger, broader, ventral sclerite; secondary suture indistinct; first metapleural pit present, second pit absent. Propodeum sharply truncate, rounded to angular, forming together with the scutellum and metanotum, the flat posterior surface of thorax; propodeal angles rounded to greatly developed laterally; apical scales small, angular, forming a right angle in lateral view; orifice oval. Coxae and trochanters normal. Femora with weakly developed basal ring. Tibia 1 with one long, narrow, blade-like spur bent medially. Tibia 2 with two normal spurs. Tibia 3 with one large blade-like spur and one normal spur. Basitarsus 1 and 2 little shorter than succeeding tarsomeres; basitarsus 3 longer than succeeding tarsomeres; tarsomeres symmetrical. Wings extending beyond apex of abdomen. Basal vein ending at base of stigma, second cubital cell much higher than wide; third cubital cell much wider posteriorly than anteriorly. Hind wing with cross-vein cu-a almost parallel with M + Cu_1 apically; vein 1A distinct from cu-a, extended somewhat beyond cu-a. Hamuli 6 to 9.

Abdomen. Spheroid, often wider than long; as wide or wider than thorax; pedicel very short. Segment II very small, cap-shaped, often flattened onto segment III; sternum II reduced, much wider than long. Segment III very large, considerably wider than long; either flattened anteriorly and abruptly curved in profile or evenly rounded. Succeeding 3 segments often retracted into segment III, very short, about 3 times as wide as long. Tergum and sternum VII rounded apically. Sting with lateral serrate margins apically.

Coloration. Very variable, ranging from entirely black to almost entirely yellow (Figs. 68, 69).

Pubescence. Variable, from very sparse and short to dense and long.

MALE. As in female except for following:

Head (Figs. 3, 4, 7). Vertex more convex. Gena much narrower than eye in lateral view. Clypeus longer, less convex. Anterior tentorial pits far below antennal sockets. Subantennal suture present. Antenna with scape relatively shorter, about .2 as long as flagellum; basal flagellomere shorter than succeeding two flagellomeres.

Thorax. Pronotum, scutellum, and propodeum more rounded than in female.

Abdomen. Apex of tergum VIII and sternum VIII + IX broadly rounded; spiculum variable; apodeme of sternum VIII broad, rounded.

Genitalia (Figs. 17, 18). Basal ring curved caudad medially. Paramere 2 to 3 times as long as high, more or less truncate apically, bearing a long parameral spine which arises from inflection of dorsal margin of paramere; ventral, mesal margin with variable emargination at base of volsellar plate and at base of paramere. Volsellar plate bearing variable, fingerlike volsellar lobe. Cuspis appressed against mesal surface of paramere. Digitus short, thick set, with large distal lobe; lobe variable. Aedeagus with variable distal lobe formed by ventrally inflected margins of spatha; apex of lobe membranous; lobe open ventrally; spatha variable; ventral hook more or less developed; aedeagal apodeme large, variable, terminating at ventral hook.

Coloration. Males are more extensively marked with yellow than females.

KEY TO THE SPECIES OF *Brachygastra*

1. Females: Antenna with 10 flagellomeres. Gena about as wide as or wider than eye in lateral view 2
   Males: Antenna with 11 flagellomeres. Gena much narrower than eye in lateral view 18
2. Height of occipital carina on ventral half of gena greater than height on dorsal half (Fig. 47) 3
   Height of occipital carina on ventral half of gena equal to or less than height on dorsal half (Fig. 2) 8
3. Scutellum rounded in profile; posterior surface not distinct from dorsal surface. Metanotum with median, dorsal, pointed projection (Fig. 65) (western South America) .... *baccalaurea*
   Scutellum angular in profile; posterior surface distinct from dorsal surface. Metanotum without median dorsal projection 4
4. Heavily punctured; scutum with medium to large sized punctures separated by about one diameter or less medially 5
   Not so heavily punctured; scutum with small to large sized punctures separated by two to three diameters or widely spaced medially 6
5. Propodeal angle greatly developed, strongly projecting laterally; distance from spiracle to apex of propodeal angle about equal to distance from apex of angle to apical scales of propodeum (Fig. 66) (Amazon Basin) .... *buyssoni*
   Propodeal angle not greatly developed, not strongly projecting laterally; distance from spiracle to apex of propodeal angle considerably less

than distance from apex of angle to apical scales (Central and South America) .... *smithii*

6. Scutum with large punctures separated by two to three diameters medially. Surface finely rugulose, not shiny 7
Scutum with scattered small to medium sized punctures widely spaced medially. Surface smooth, shiny (southern Brazil and southwestern Amazon Basin) .... *bilineolata*

7. Yellow markings often extensive on propodeum. Scutal lines strongly narrowed or interrupted medially (southwestern Amazon Basin) .... *propodealis*
Yellow markings never extensive on propodeum. Scutal lines of even width, never interrupted medially (Central America) .... *smithii*

8. Scutellum angular; dorsal surface flat or slightly convex; margin between dorsal and lateral surfaces angular (Figs. 9, 14) 15
Scutellum rounded; dorsal surface moderately to strongly convex; margin between dorsal and lateral surfaces rounded (Figs. 25, 26) 9

9. Pronotal keel well developed 10
Pronotal keel absent (Mexico) .... *azteca*

10. Pronotal keel extending onto lateral surface of pronotum as an acute or rounded ridge (Fig. 62) 13
Pronotal keel absent or indistinct on lateral surface of pronotum (Fig. 40) 11

11. Abdomen with very large punctures separated by one diameter or less. Surface of tergum 2 rugose, dull. Tergum 2 little wider than long (Fig. 69) 12
Abdomen with small punctures separated by about two diameters. Surface of tergum 2 smooth, shiny. Tergum 2 distinctly wider than long (Central and South America) .... *augusti*

12. Posterior surface of scutellum low, about 7 times as wide as height at middle (Fig. 34); surface irregularly concave (southern Brazil) .... *fistulosa*
Posterior surface of scutellum higher, about 4 times as wide as height at middle (Fig. 42); surface flat (Central and South America) .... *scutellaris*

13. Abdomen with small punctures. Surface of tergum 2 smooth. Tergum 2 much wider than long 14
Abdomen with very large punctures separated by one diameter or less. Surface of tergum 2 rugose. Tergum 2 little wider than long (Fig. 69) (Central and South America) .... *scutellaris*

14. Dorsal surface of scutellum distinctly sloping dorsad, scutellum projecting as high as surface of scutum or above (Fig. 58). Axillar ridges strongly swollen, almost spheroid. Surface of tergum 2 dull, with small punctures separated by about one diameter (central South America) .... *smithii*
Dorsal surface of scutellum not distinctly sloping dorsad, scutellum not projecting as high as surface of scutum (Fig. 53). Axillar ridges

moderately swollen, ovoid. Surface of tergum 2 shiny, with very small punctures separated by about two to three diameters (northern South America and Trinidad) .... *bilineolata*

15. In dorsal view posterior margin of scutellum distinctly emarginate, V-shaped, lateral length of scutellum as much as 1.2 times as long as median length (Fig. 12); in lateral view distinctly projecting over plane of metanotum (Figs. 14, 15). (Panama and South America) ...... *lecheguana*
In dorsal view posterior margin of scutellum straight to slightly curved or V-shaped; in lateral view only slightly projecting over the plane of the metanotum 16

16. Heavily punctured; metanotum rugose, with irregular punctures, convex (Fig. 11). Hairs on vertex at least two times as long as width of ocellus. Yellow markings much reduced or lacking (southern Andes) .... *borellii*
Not so heavily punctured; metanotum smooth, punctures, when present, restricted to dorsal margin; metanotum flat. Hairs on vertex about as long as width of ocellus. Yellow markings always present 17

17. *South America .... *lecheguana*
Mexico and Central America .... *mellifica*

18. Spatha abruptly expanded between ventral hooks and aedeagal lobe (Fig. 55) 19
Spatha not expanded between ventral hooks and aedeagal lobe (Fig. 17) 21

19. In ventral view aedeagal lobe present, distinctly wider than median width of spatha 20
In ventral view aedeagal lobe not visible, apex of aedeagus not at all widened (Fig. 29) .... *azteca*

20. In ventral view digital lobe extending beyond apex of cuspis (Fig. 55). Aedeagal lobe evenly rounded .... *bilineolata*
In ventral view digital lobe not extending to apex of cuspis (Fig. 63). Aedeagal lobe moderately tapered to a rounded apex .... *smithii*

21. Aedeagal lobe narrowed at base 22
Aedeagal lobe distinctly wider at base than at apex (Fig. 37) .... *augusti*

22. In lateral view apex of digital lobe blunt, forming a right angle (Figs. 18, 21) .... *lecheguana*
In lateral view apex of digital lobe produced into a long pointed projection (Fig. 24) .... *mellifica*

SPECIES ACCOUNTS

Most of the species of the genus can be placed in either of two species groups, the *smithii* group or the *lecheguana* group, but four species, *azteca, augusti, fistulosa,* and *scutellaris* cannot be placed in either of these groups and are here placed in an undefined group.

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* Because of the lack of reliable diagnostic characters in these species, locality is used here. See the discussion of variation in these species below.

Brachygastra augusti is similar to the lecheguana group in its color pattern, but has the large pronotal keel like *scutellaris*. *B. scutellaris* has a color pattern similar to that of the *smithii* group, but is morphologically quite distinct. *B. fistulosa* is intermediate between *scutellaris* and *augusti*. *B. azteca* is like the former species on the basis of the rounded propodeum and the punctation, but is unlike all other species in the absence of the pronotal keel.

Brachygastra azteca (Saussure) new combination
(Figs. 25-30, 33)

Nectarinia azteca Saussure, 1857. Rev. Mag. Zool. 9:280 (♀, Cuautla, Morelos, Mexico; lectotype in Musée National d'Histoire Naturelle, Paris, by present designation).

Nectarina azteca: Dalla Torre, 1904. Gen. Insect., fasc. 19:86.

Chartergus mexicanus Cameron, 1906. Invertebrata Pacifica 1:154 [♀, Santiago de las Vegas; in the British Museum (Natural History)].

This small species is restricted to Mexico, where it is common in the southern and western states. *Brachygastra azteca* resembles *scutellaris* both in size and general body form. Like *scutellaris*, *azteca* has a distinctly rounded scutellum and propodeum and heavily punctured abdominal segments. It differs from *scutellaris* and other species of the genus, however, by the rounded pronotum which has neither a well developed pronotal keel nor a prominent humeral angle. The pronotum could be said to resemble that found in *baccalaurea* but the latter species never loses the keel entirely as in *azteca*. This species can be best recognized by the rounded abdomen which lacks the flattened dorsolateral surfaces and the abruptly convex anterior surface found in many of the other species. In addition, the first abdominal segment is distinctly narrower than the second giving the abdomen a petiolate appearance. The second tergite bears a wide, yellow, apical band which has two anterolateral emarginations. This band, unlike that of other species is quite stable and perhaps is the best single character for easy recognition of the species.

FEMALE. (1.) Wing length 5.93 ± .247 mm.

Head. (2.) In frontal view .87 times as high as wide; in dorsal view .36 times as long as wide; posterior margin strongly curved. (3.) Lateral ocellus separated from eye by about 1.41 times distance between lateral ocelli and from occipital carina by about 1.14 times this distance; punctures dense, medium sized, separated by one diameter or less, often contiguous; vertex very strongly convex, posterior surface sloping strongly ventrad in profile. (4.) In lateral view gena about .75 times as wide as eye at middle; postgenal convexity slight; gena about 0.9 times as wide at level of eye emargination as at level of convexity; punctures medium sized, unevenly spaced dorsally, separated by one diameter or less, often forming long rows, smaller, more scattered ventrally. (5.) Occipital carina low, acute, uniform in height, extending to middle of postgenal convexity. (6.) Frons with large, deep, punctures, evenly spaced, separated by about one half diameter, often contiguous. (7.) Clypeus about 1.7 times as wide as long, moderately convex; distal margin straight, broadly rounded onto lateral lobe; apical triangle about as long as width of antennal socket, apex broadly rounded; epistomal suture forming about a 45° angle with eye margin, dorsally evenly curved; surface smooth, basal 0.7 of clypeus sericeous; punctures very small, sparse. (8.) Malar space small, less than 0.3 width of antennal socket. (9.) Antenna with flagellum strongly swollen, eighth flagellomere about 2.2 times as wide as long. (10.) Head with abundant, short, erect, white hairs slightly less than width of ocellus in length, slightly longer on vertex; eyes with dense short erect hairs; head very lightly sericeous.

Thorax. (11.) Anterior surface of pronotum with scattered small to medium sized punctures dorsolaterally, very small or absent medially; anterior surface not distinctly separated from dorsal surface, in profile rounded onto dorsal surface, not forming a distinct angle with dorsal surface; pronotal keel absent; humeral angle rounded, forming a low, blunt, prominence; dorsal surface evenly rounded onto lateral surface, with deep, medium sized punctures contiguous or nearly so dorsally, smaller and more scattered laterally; lateral surface very wide, with medium sized punctures separated by about one diameter; pronotal lobe distinct, wide. (12.) Scutum about .85 times as long as wide; punctures medium to large, separated by one diameter or less, often contiguous. (13.) In dorsal view scutellum about 3 times as wide as long, posterior margin straight; in posterior view about 3 times as wide as high, dorsal margin straight; in profile evenly rounded, low, not prominent; dorsal surface evenly convex, anterior margin of convexity with small median emargination, dorsal surface rounded onto slightly flattened posterior surface; scutellar pocket well developed, concave, smooth; dorsal surface with medium sized punctures separated by one diameter or less extending onto dorsal portion of posterior surface; axillar ridges prominent, not greatly swollen, with few small punctures. (14.) Metanotum about 2.6 times as wide as long, slightly concave medially; dorsal margin evenly bowed dorsad, forming a slight lip medially; ventral margin very slightly V-shaped; surface smooth with few fine punctures laterally. (15.) Mesopleuron strongly convex; anterior and posterior surfaces with scattered, small punctures; punctures contiguous, medium sized dorsally, slightly smaller, separated by two diameters or less, ventrally. (16.) Dorsal sclerite of metapleuron about twice as high as wide at middle with scattered small punctures; secondary suture indistinct; first metapleural pit small, shallow; ventral sclerite smooth; metapleural-propodeal suture evident as distinct furrow. (17.) Propodeum rounded, swollen; posterior surface with distinct, narrow deep, median concavity, surface rugulose medially, heavily rugose or punctured dorsolaterally; lateral surface with large punctures or rugose sculpturing posteriorly, small irregular punctures anteriorly; lateral ridge low, indistinct, irregular; propodeal angle not well developed, swollen, rounded in lateral and posterior views, occasionally forming rounded obtuse angle. (18.) Thorax with short white hairs in punctured areas, longer on propodeum than elsewhere; thorax lightly sericeous.

Abdomen. (19.) Tergum 1 cap-shaped, distinct from tergum 2; about 3.5 times as wide as long in dorsal view; sternum 1 about 3 times as wide as long; tergum 1 with scattered small punctures, sternum finely rugulose. (20.) Tergum 2 about .85 times as long as wide, low, evenly convex in profile, with deep, medium to large sized punctures separated by two diameters or less, fewer and smaller anteriorly; sternum 2 with punctures as on tergum. (21.) Terga and sterna 3-5 with deep, medium sized punctures separated by one to two diameters. Tergum and sternum 6 with few fine punctures. (22.) Abdomen with sparse, short, white hairs.

Coloration. Black with yellow markings as follows: Two small interantennal spots; apex of clypeus; lower half of inner orbit; band on ridge of pronotum; posterior apices of pronotum; two posterolateral scutellar spots; dorsal and lateral margins of metanotum; apical band of tergum 1; broad apical band with small, lateral, anterior emarginations on terga and sterna 2-6. Flagellum slightly fulvous ventrally. Apex of clypeus and mandible, coxae and legs dark brown to ferruginous.

MALE. (1.) Wing length 5.89 ± .577 mm.

As in female except for following:

Head. (2.) In frontal view about .85 times as high as wide; in dorsal view about .32 times as long as wide. (3.) Lateral ocellus separated from eye by .93 times distance between lateral ocelli and from occipital carina by .87 times this distance. (4.) Gena about .3 times as wide as eye in lateral view; postgenal convexity lacking; punctures medium sized on entire surface of gena. (7.) Clypeus about 1.34 times as wide as long; slightly convex; distal margin straight; contact with eye about twice width of antennal socket; epistomal suture forming about a 30° angle with eye; apical triangle about as long as width of antennal socket; clypeus entirely sericeous. (8.) Malar space very short. (9.) Scape about .6 width of clypeus; flagellum moderately swollen, eighth flagellomere 1.7 times as long as wide. (10.) Head heavily sericeous.

Thorax. (13.) Dorsal surface of scutellum strongly convex, anterior margin of convexity higher than posterior margin.

Abdomen. (24.) Spiculum long, narrow, about three times as long as width at base, evenly tapered to a rounded apex.

Genitalia. (25.) Paramere about twice as long as high; apex truncate, rounded; parameral spine long, about half as wide at middle as at basal inflection; paramere with small, shallow notch at base of volsellar plate.

(26.) Volsellar lobe depressed, in ventral view about .5 times as wide at base as long, extending to middle of digital lobe, evenly tapered to a blunt apex. (27.) Cuspis flattened against paramere, pointed in lateral view, with few small black teeth opposite base of digitus. Digitus stout, in lateral view about twice as long as width at base, posterior angle of digital lobe blunt, rounded, anterior angle acute, slightly rounded, directed slightly ventrad; in ventral view posterior angle acute, rounded, with dense, short erect hairs, anterior angle blunt, rounded; mesal surface of lobe with few scattered small punctures; lateral surface with a curved band of small black teeth extending from anterior angle to base of digitus, teeth larger basally than apically. (28.) Aedeagus in lateral view slightly curved ventrad, lobe about .45 length of entire spatha, lobe flat, slightly swollen; in ventral view lobe as wide at apex as at base, slightly narrower than middle of spatha, with ventral inflected margins parallel on basal 0.5 of lobe, meeting at middle; spatha abruptly widened at base of lobe to about 1.6 times width of spatha at middle, with lateral row of small teeth basally; ventral hook long, curved laterad apically; in lateral view aedeagal apodeme wide, slightly angular, widened strongly about 0.3 distance from base to hook, forming blunt right angle ventrally; in dorsal view, compressed.

Coloration. Black with yellow markings more or less developed as follows: two interantennal spots or entire interantennal area; innerorbits extending as far dorsad as eye emargination; apical margin to entire clypeus; ventral surface of scape and occasionally pedicel; pronotal ridge; posterior apices of pronotum; median and lateral spots on scutellum; dorsal margin of metanotum; anterior and posterior spots on tegula; ventral surfaces of coxae and trochanters; apical band on metasomal tergum 1; apical bands on metasomal terga and sterna 2-6, sternal bands with lateral emarginations. Flagellum ferruginous ventrally. Wings clear with dark brown veins.

Type Material. There are three female syntypes in the Musée National d'Histoire Naturelle, Paris, labeled "Nectarinia azteca Sauss., cotype. H. de Saussure det." These are, no doubt, of the original type series but it is possible that they do not represent the entire series. Labels on two of the specimens indicate that Saussure gave them to Sichel in 1867. There are twelve additional specimens in Saussure's collection in Geneva which probably represent the remainder of the specimens he examined in 1857.

Two of the syntypes are labeled "Cuantla, t.c." which probably refers to Cuantla, Morelos. I have designated one of these as lectotype. It has the following labels: "Museum Paris, Mexique, Coll. O. Sichel 1867"; "Cotype!"; "Cuantla, t.c."; "Cotype donné a Sichel par Saussure"; "Nectarinia azteca Sauss., Mexique"; "Nectarinia azteca Sauss., Mexique"; "Nectarinia azteca Sauss., cotype, H. de Saussure det."

VARIATION. The color pattern of *azteca* varies somewhat geographically but never approaches the extremes of variation as found in the South American species. Likewise, in strong contrast to the other species, the variation at any one locality is slight.

Specimens from Sinaloa and Sonora commonly display the fully developed color pattern as described above. There is a gradual decrease in head and thoracic color pattern toward the south, and specimens from Chiapas, the southernmost extremity of the range, have only the inner orbital spots and occasionally a small pronotal spot. Intermediate specimens, i.e., those with orbital spots and a well developed band on the pronotum, are found in Jalisco, Michoacan and Nayarit. The most persistent markings are the orbital spots, which, even though reduced, never disappear, and the abdominal bands, which vary little throughout the range.

DISTRIBUTION. *Brachygastra azteca* is the only species of the genus that is restricted to Mexico. It appears to be restricted to the west coast and south central Mexico.

Specimens have been examined from the following localities. *Chiapas:* 5 mi. E., 28 mi. W. of Cintalapa; Comitán; El Ocotál; 1 mi. S., 2 mi. N. of Suchiapa; 6 mi. N. of Villa Flores. *Chihuahua:* 2 mi. S. of Matáchic. *Colima:* 5 mi. W. of Manzanillo. *Guerrero:* 40 mi. N., 20 mi. E., 9 mi. W. of, and Acapulco; Chilapa; 3 mi. N. 4000 ft., 16 mi. N., 5 mi. S. 2 mi. E. of Chilpancingo; Hacienda de la Imagen 4000 ft.; 1.5 mi. W. Mochitlán; 23 mi. N.E., 19.5 mi. N.E. of, and Taxco; Xalitla 1500 ft. *Guanajuato:* Guanajuato; 25 mi. S.W. Salvatierra. *Jalisco:* Chapala; 18 mi. S. of, and Guadalajara; 15-20 mi. W. of Jiquilpan; Lagos de Moreno, 6400 ft.; 5 mi. S.E. of Plan de Barrancas. *Mexico:* 5 mi. N., 15 mi. S. of, and Ixtapan de la Sal 5500 ft.; 13.5 mi. S.E. of Tenancingo; 6 mi. W. of Zautepec. *Michoacán:* 11 mi. E. of Apatzingán; Chavinda 5800 ft.; 6 mi. W. of Jacona; 2 mi. S. of Tziztio 4450 ft.; 15 mi. E. of Zamora. *Morelos:* 3 mi. N. 3400 ft., 2 mi. S. 3000 ft. of, and Alpuyeca; Canyón Lobos [4.3 mi. W. Yautepec]; 4 mi. E. 6000 ft., 3 mi. N.W. Cuernavaca 5500 ft.; Huajintlán 2800 ft.; 6 mi. S.W. Jonacatepec 3700 ft.; Lake Tequesquitengo 2800 ft.; Matamoros; Tetecala 3500 ft.; 6 mi. S. Temixco; Tepotzlán; 7 mi. N.W. 4000 ft. of, and Yautepec. *Nayarit:* Ahuacatlán; San Blas; 6 mi. S. Temixco; 13 mi. S.W. of, and Tepíc 3000 ft. *Oaxaca:* 20 mi. E. of El Camarón; 48 mi. S. of Chivela; 7 mi. N.E. of, and Juchitán; 23 mi. S. Matías Romero; 47 mi. S.E. Oaxaca; (Sierra de Pluma); Salina Cruz; 8 mi. N.W. of Tamazulapan 6500 ft.; 64 mi. W., 48 mi. W., 44 mi. W., 14 mi. N.W. 700 ft. of, and Tehuantepec; 12 mi. N.W. Totolapan; Zanatepec. *Puebla:* 11 mi. S.E. of Acatlán; 7 mi. N. of Izúcar de Matamoros 4450 ft.; 2 mi. N.W. Petlalcingo 4600 ft.; Tehuacán; 8 mi. S.E. 4100 ft. of, and Tehuitzingo. *Sinaloa:* 13 mi. N., 20 mi. S. 250 ft. of Culiacán; 14 mi. S.E., 8 mi. S.E. of Elota; 16 mi. S. of, and Guamúchil; 1 km. N.W. St. Lucia 3700 ft. *Sonora:* La Aduana; 7 mi. W. of, and Alamos; (Bakachaka) on the Río Mayo; Cócorit; Esperanza; San Bernardo on Río Mayo.

BIOLOGY. Although *B. azteca* is very common in many parts of Mexico, relatively little is known about its biology. According to Buysson (1905), the nests are constructed in shrubs and cacti, but I have seen them in large trees as well. The nest is the spherical phragmocyttarous type of Saussure, *i.e.*, the combs are strongly convex and placed one below the other as in *Polybia*. The communicating passageways in the combs are lateral but are not placed one below the other as is common in most other phragmocyttarous nests. Buysson reported that there may be two or more openings in one comb.

M. L. Diguet collected three nests of *azteca*, the largest of which contained nine combs and was 12 cm. long and 15 cm. wide. One of these nests (Buysson, 1905, Pl. 14, fig. 2) had the carton formed into a lateral, tube-shaped entrance at the lowest comb, a structure not found in any other *Brachygastra* and resembling that found in some *Parachartergus* nests.

B. azteca is known to store honey in its nests but the nests are not collected for it as are those of *mellifica*.

I have seen *azteca* as a common visitor on many flowers, especially Leguminosae. It has also been collected on *Asclepias, Croton, Donnelsmithia* and *Solanum.* It is attracted in large numbers to sweet juices of ripe fruit.

Brachygastra scutellaris (Fabricius)
(Figs. 42-45)

Vespa scutellaris Fabricius, 1804. Syst. Piezat., p. 265 (2 ♀♀, South America; lectotype in Universittetes Zoologiske Museum, Copenhagen, by present designation).
Brachygastra scutellaris Perty, 1833. Delectus Animalium Articul. Brazil., p. 146 (no type or locality given).
Chartergus scutellaris; Möbius, 1856. Abhandl. Naturw. Ver. (Hamburg) 3:143, 144, pl. 15.
Brachygastra scutellata Spinola, 1851. Mem. Acad. Sci. Torino 13:74 (4 ♀♀, Brazil; in the Museo di Zoologia, Torino).
Nectarinia rufiventris Saussure, 1853-1858. Et. Fam. Vesp. 2:226 [♀, Pará, Brasil; in the British Museum (Natural History)].
Nectarinia scutellata; Saussure, 1853-1858. Et. Fam. Vesp. 2:226, 227, 231, 234.
Nectarina rufiventris; Smith, 1857. Cat. Hymen. Brit. Mus. 5:136.
Nectarina scutellata; Smith, 1857. Cat. Hymen. Brit. Mus. 5:136.
Nectarina scutellata var. *rufiventris;* Ducke, 1904. Bol. Mus. Goeldi 4:322.
Caba rufiventris; R. von Ihering, 1904. Rev. Mus. Paulista 6:106, 108-109.
Nectarinia scutellata var. *gribodoi* Buysson, 1905. Ann. Soc. Ent. France 74:549 (♀♀, "Haut Amazone" and Iquitos, Perú).
Nectarinia scutellaris; Ducke, 1905. Rev. Ent. (Caen) 24:11.
Brachygastra scutellaris var. *myersi* Bequaert, 1942. Jour. New York Ent. Soc. 40:308 (♀ holotype and 5 ♀♀ paratypes; Mt. Roraima, British Guiana, ♀ paratype, Cavinas, Río Beni, Bolivia; in the Museum of Comparative Zoology, Harvard).
Brachygastra scutellaris var. *annectens Bequaert,* 1942. Jour. New York Ent. Soc. 50:307 (♀ holotype and ♀ paratype, Muzo, Dept. Boyaca, 900 m, Colombia; in the Museum of Comparative Zoology, Harvard).
Brachygastra scutellaris var. *colombiensis* Bequaert, 1942. Jour. New York Ent. Soc. 50:308 (♀ holotype and 4 ♀♀ paratypes, Restrepo, Dept. Meta, Colombia; in the Museum of Comparative Zoology, Harvard).
Brachygastra scutellaris var. *gribodoi;* Bequaert, 1942. Jour. New York Ent. Soc. 50:307-308.
Brachygastra scutellaris var. *rufiventris; Bequaert,* 1942. Jour. New York Ent. Soc. 50:307.

As is evident in the above list, this species is quite variable throughout its range and five forms or varieties have been described. It is the smallest species of the genus (5-6 mm., h + th + terg 2) and is quite distinct in the very heavily punctured thorax and abdomen, a character which Fabricius recognized in his original description.

The most common form of the species is easily recognized by the brilliant yellow scutellum and metanotum and the pale, narrow, apical bands on the abdominal terga (b, Fig. 69). On the basis of color alone, the darker forms, which occasionally lack maculations entirely, could be confused with *augusti* and *fistulosa* and the lighter forms extensively marked with yellow with *smithii, bilineolata* and *propodealis*. *B. scutellaris* is easily distinguished from these species by the following morphological features: the propodeal angles are swollen and rounded as in *azteca* (Fig. 28), the lateral ridge of the propodeum is weak or absent, the second abdominal segment is only a little wider than long (Fig. 69) and is flattened dorsolaterally, and the abdomen is covered with very large deep punctures giving the cuticle a dull appearance. Hairs are very sparse and in many areas may be lacking entirely.

FEMALE. (1) Wing length 5.07 ± .852 mm.

Head. (2.) In frontal view .85 times as high as wide; in dorsal view .45 times as long as wide; posterior margin moderately curved or roundly V-shaped. (3.) Lateral ocellus separated from eye by 1.5 times distance between lateral ocelli and from occipital carina by 1.6 times this distance; vertex with large, deep punctures separated by one diameter or less; vertex flat. (4.) In lateral view gena about 0.8 times as wide as eye at middle; postgenal convexity slight; gena about 1.4 times as wide at level of convexity as at level of eye emargination; punctures large, almost contiguous mediodorsally, smaller, more scattered ventrally, very small at level of convexity. (5.) Occipital carina low, acute dorsally, extending to postgenal convexity, indistinct and rounded on convexity. (6.) Frons with medium sized, deep, punctures separated by about one diameter. (7.) Clypeus about 1.7 times as wide as long; moderately convex; distal margin straight, broadly rounded onto lateral lobe; apical triangle about 1.5 times as long as width of antennal socket, apex narrowly rounded; contact with eye equal to about width of antennal socket; epistomal suture forming about a 60° angle with eye margin, dorsally forming a broad V; surface smooth, shiny, basal 0.5 sericeous, with few fine punctures. (8.) Malar space 0.3 times as long as width of antennal socket. (9.) Antenna with flagellum strongly swollen, eighth flagellomere about twice as wide as long. (10.) Head with very sparse, short, erect, yellow hairs; eyes almost bare, with very short hairs; head lightly sericeous.

Thorax. (11.) Anterior surface of pronotum smooth, without punctures, distinctly separated from dorsal surface; pronotal keel well developed, extending to level of pronotal lobe, keel rounded and high medially, forming strong dorsolateral anterior inflection at humeral angle, becoming broad and rounded ventral to angle, inflection little wider than width of ocellus; humeral angle rounded in dorsal and lateral view, overlapping occipital carina when head is flexed; dorsal surface of pronotum evenly curved onto lateral surface, with large, deep, punctures, contiguous or nearly so; lateral surface very narrow, rugose; pronotal lobe distinct, wide. (12.) Scutum about .75 times as long as wide; punctures large, deep, separated by one diameter or less, often contiguous. (13.) In dorsal view scutellum 3 times as wide as long, slightly bilobed, posterior margin slightly curved medially; in posterior view about 4.5 times as wide as high at middle; in profile dorsal margin not extending over plane of metanotum; dorsal surface evenly, moderately convex, posterior surface flattened, curving gradually onto dorsal surface; scutellar pocket distinct, concave anteriorly, flattened posteriorly; dorsal surface with medium sized, deep, contiguous punctures; posterior surface with medium sized punctures dorsally; axillar ridge rounded, small, closely associated with scutum. (14.) Metanotum 3 times as wide as long at middle, very slightly concave medially; dorsal margin evenly bowed slightly dorsad, ventral margin slightly curved, almost straight; smooth without punctures. (15.) Mesopleuron strongly convex; anterior and posterior surfaces smooth with few small punctures; punctures large, separated by two diameters or less medially. (16.) Dorsal sclerite of metapleuron about 3.5 times as high as wide at middle, rugose; secondary suture indistinct; first metapleural pit deep, furrow shallow; ventral sclerite smooth, with few scattered small punctures; metapleural-propodeal suture not evident. (17.) Propodeum rounded; posterior surface with small, round, deep, central concavity; concavity smooth, dorsolateral surface of posterior surface with large deep contiguous punctures; lateral surface of propodeum with large, deep, contiguous punctures posteriorly, smaller, scattered anteriorly; lateral ridge rugose, low; propodeal angles rounded in profile; propodeum swollen posterolaterally in caudal view. (18.) Thorax very lightly sericeous, almost bare, with few scattered short erect white hairs, more abundant on posterior surface.

Abdomen. (19.) Tergum 1 cap-shaped, distinctly separate from tergum 2, about 4 times as wide as long in dorsal view; sternum 1 about 5.3 times as wide as long; tergum with scattered small punctures, sternum rugose. (20.) Tergum 2 about .84 times as wide as long; somewhat flattened dorsolaterally; in profile flattened anteriorly, rounded abruptly onto dorsal surface, somewhat constricted apically; punctures large, separated by one diameter or less, with shallow furrows posteriorly; sternum 2 with large punctures separated by about two diameters or less. (21.) Terga 3-6 and sterna 3-5 rugose, with small punctures posteriorly; sternum 6 smooth with few fine punctures distally. (22.) Abdomen bare except for few scattered erect short hairs.

Coloration. Entirely black to black with yellow markings more or less developed as follows: Inner orbits; apical margin of clypeus; dorsal and ventral medial spot on gena; broad V-shaped marking on vertex; dorsal surface of pronotum; tegula; two parallel lateral lines on scutum; scutellum; axillar ridges; subtegular spot on mesopleuron; metanotum; median concavity of posterior surface of pronotum; apical band on tergum 1; entire surface of tergum 2; broad apical bands on terga 3-5 and sterna 2-5. Mandible, flagellum, legs dark brown. Wings black.

MALE. Ducke (1904) mentions the male but to my knowledge it has never been adequately described and I have seen none.

Type Material. Two specimens labeled "V. scutellaris, ex. Amer. mer.-Schmid." in the Schestedt-Tonderlund collection at the Universittetes Zoologiske Museum in Copenhagen bear "Type" labels and are apparently Fabricius' types. One of these has been labelled lectotype by R. M. Bohart (unpublished) and I hereby record this designation.

I have seen paratypes of Bequaert's varieties and found no differences other than color between them and the specimen compared with the Fabrician types.

Variation. The structural characters of *B. scutellaris* do not differ markedly geographically. The color pattern is quite variable throughout the range and at many localities. The most common form has a yellow scutellum and metanotum and pale, narrow apical bands on the abdominal segments (= var. *gribodoi* Buysson) (b, Fig. 69). It occurs throughout the range and is the only form I've seen from Perú. A somewhat less common form like the above but with wider abdominal bands (= typical *scutellaris*) is found in Honduras and British Guiana. Specimens in which the yellow on the scutellum and metanotum is more or less reduced and in which abdominal bands are very narrow (= *myersi* Bequaert) are common in British Guiana and also occur in Bolivia, Brazil and Ecuador. Entirely black specimens (= *colombiensis* Bequaert) (a, Fig. 69) have been seen from Colombia (Dept. Meta) and Brazil (Acre and Guaporé). At the opposite extreme are forms almost entirely yellow which have been collected in Colombia (Dept. Boyaca) (d, Fig. 69). This color variation is continuous, and many individuals fall between the situations outlined above. Saussure described *Nectarinia rufiventris* from Brazil on basis of the brown pigmentation of the second tergite. Richards and Richards (1951) have found this to be a condition in which the reddish color of the teneral specimen persists into the adult.

In view of the absence of discrete morphological differences and distinct geographical relationships between the above forms they must be considered one species. Additional information and specimens should clarify the situation. Analysis of many nests populations would add much to the understanding of the species. Richards and Richards have found, in their examination of *scutellaris* nests in British Guiana, that the extent of yellow color was correlated to some extent with ovarial development, *i.e.* caste, but a definite relationship could not be demonstrated without additional nest studies.

Distribution. *B. scutellaris* ranges from Honduras to southern Brazil (Rio de Janeiro). Throughout its range it is sympatric with *augusti* and *smithii*, but it does not extend into Paraguay and Argentina. It is found as far west as the Departamento of Cajamarca in northern Perú and extends into the drier regions of northeastern Brazil (Pernambuco).

Specimens have been examined from the following localities: CENTRAL AMERICA. *Costa Rica.* Limón Prov.: Guapiles, 200 m. *Honduras.* Tela Jilamo farm. *Panama.* Barro Colorado Island; Cabina; Summit.

SOUTH AMERICA. *Brazil.* Est. Acre: Iquiri. Est. Guanabara: Rio de Janeiro. Est. Guaporé: Porto Velho. Est. Mato Grosso: Chapada; West Border. Est. Pará: Obidos. Est. Pernambuco: Varyea. *British Guiana.* Essequibo Co.: Bartica; Kaieteur, Savanna; Mazaruni Station; Mt. Roraima. *Bolivia.* Dept. Beni: Blancaflor; Cavinas; Huachi; Reyes; Rurrenabaque 175 m. Dept. Cochabamba: Cristal Mayu; Chapera 200 m. Dept. Pando: north of Mapirí River on Río Beni. *Colombia.* Dept. Boyaca: Muzo. Dept. Meta: Restrepo. *Ecuador.* Napo. *French Guiana.* Dept. Guyane: Cayenne; Noveau Chantier; St. Jean du Maroni. *Peru.* Dept. Cuzco: Maracapata; Valle del Río Cusnipata (= ?Cusipata); Santa Isabel. Dept. Huánuco: Monzon Valley; Tingo María; Río Huallaga 670 m. Dept. Junin: Colonia Perené, 18 mi. N.E. La Merced; Satipo, 700 m. Dept. Loreto: Iquitos; Pevas. Dept. Pasco: Río Aguashiri; Cam. del Pichis. *Surinam.* Dist. Marowijne: Albina.

BIOLOGY. The nest of *B. scutellaris* has been described and figured by Moebius (1856, as *Chartergus*), Ducke (1907) and Rau (1933). The nest described by Rudow (1889) is not of *Brachygastra.* The nest is pyriform and has a single, oval, lateral entrance which opens into the space between the lowest comb and the carton. The largest nest that has been described (Richards and Richards, 1951) was 10 cm. long by 7.5 cm. wide and contained 6 combs and 876 wasps. Van der Vecht (label data) found a nest in the initial stages of construction which contained 62 wasps. The fragile envelope often is streaked with white. The cells are relatively shallow, and the pupae extend considerably beyond the cell.

In British Guiana, the species is associated with clearings or open scrubby woodland but I have seen it in virgin rain forest in Costa Rica.

Richards and Richards dissected samples of two nest populations of 863 and 876 wasps. They found these to contain 8% queens and 30% intermediates, and 6.5% queens and 43.5% intermediates, respectively. Intermediates had ovaries "more or less developed" but distinctly intermediate between the condition found in the workers and queens. Both the extent of the yellow pigmentation and the number of hamuli appeared to be correlated with ovarial development. Both nests were in a similar state of development with large numbers of all stages present, and it is possible that the nests are relatively long lived but probably are not perennial as are those of *lecheguana.*

Brachygastra fistulosa new species
(Figs. 31, 32, 34)

Records of *B. augusti* var. *quinta* from southern Brazil refer to this species.

Among specimens of *Brachygastra augusti* from southern Brazil, I found eight that differ distinctly from other examples from the area and from other species in the genus. While differing in head, ocular, and abdominal proportions, an even more striking deviation from typical *augusti* is the abundance of very large punctures on the abdomen which are similar to those found in *scutellaris.* Additional differences in propodeal and scutellar characters were also noted. Although *fistulosa* occurs together with *augusti,* no intermediate conditions of the above characters were noted and I feel certain this is a distinct species.

R. von Ihering's variety, *quinta*, was also described as having "punctuation trés intense et trés grosse" on the second abdominal tergite, but these punctures are of a distinctly different character being smaller, deeper, and very dense. His specimens from Rio Jurua collected by Garbe in 1902 have been examined and they undoubtedly are distinct from *fistulosa*. His additional reference to specimens of *quinta* from the states of Paraná and São Paulo, however, is probably a reference to *fistulosa*, the only *Brachygastra* "entierement noir, vu par dessus" that I have seen from those states.

Although *fistulosa* resembles *augusti* very closely, examination of the following characters will serve to distinguish it from the latter as well as from other species in the genus. The scutellum is low but retains a rectanguloid shape similar to that found in *lecheguana*, a much larger wasp. The large punctures on the abdomen resemble only those found in *scutellaris* and give the cuticle of both these wasps a dull appearance. *B. fistulosa* can be distinguished from *scutellaris* by the proportions and shape of the second abdominal tergum. The latter has a relatively longer tergum (Fig. 69), the cross-section of which is roundly triangular, *i.e.* strongly produced dorsally. *B. fistulosa* is entirely black when viewed from above and may resemble melanic forms of the smaller *scutellaris*.

FEMALE HOLOTYPE. (1.) Wing length 6.50 mm.

Head. (2.) In frontal view .86 times as high as wide; in dorsal view .43 times as long as wide; posterior margin slightly curved. (3.) Posterior ocellus separated from eye by 1.59 times distance between posterior ocelli and from occipital carina by 1.41 times this distance; vertex with small to medium sized punctures separated by one diameter or less, often contiguous, punctures widely separated posterolateral to lateral ocellus; vertex strongly convex, posterior surface sloping ventrad in profile. (4.) Gena as wide as eye in lateral view; postgenal convexity wide, low; gena about 1.3 times as wide at level of convexity as at level of eye margination; gena with small to medium sized punctures irregularly spaced, separated by four diameters to contiguous, occasionally forming long rows, only slightly smaller ventrally, much smaller on convexity and along ventral margins. (5.) Occipital carina a low acute ridge of even height extending to the mandibular condyle. (6.) Frons with deep medium sized punctures separated by about one diameter or less, often contiguous. (7.) Clypeus about 1.7 times as wide as long; strongly convex; distal margin curved, broadly rounded onto lateral lobe; apical triangle about as long as width of antennal socket, apex very broadly rounded; contact with eye equal to width of antennal socket; epistomal suture forming an angle of about 70° with eye margin, dorsally indistinct; clypeal surface smooth, shiny, with few scattered small punctures, basal .2 sericeous. (8.) Malar space very small, about .2 times as long as width of antennal socket. (9.) Antenna with flagellum strongly swollen, eighth flagellomere about 2.1 times as wide as long. (10.) Head with short erect yellow hairs, length on vertex equal to width of anterior ocellus, shorter elsewhere; eyes with moderately dense short hairs about half as long as width of antennal socket; head very lightly sericeous.

Thorax. (11.) Anterior surface of pronotum smooth, without punctures, distinctly separated from dorsal surface by keel; pronotal keel low and rounded medially, high and distinct at humeral angle, indistinct and forming broad blunt ridge below humeral angle; humeral angle rounded, keel inflected cephalad forming a rounded collar-like extension equal to about width of ocellus at midpoint; dorsal surface of pronotum evenly rounded onto lateral surface, with medium sized punctures separated by less than one diameter or contiguous; lateral surface narrow, rugose, with irregular, small punctures; pronotal lobe distinct, wide. (12.) Scutum .83 times as long as wide; punctures medium sized, separated by about two diameters or less, slightly smaller and more scattered posteriorly. (13.) Scutellum angular, very slightly bilobed, 2.85 times as wide as long in dorsal view, in profile forming a low angular projection; dorsal surface slightly convex with large, deep, contiguous punctures, occasionally slightly separated; posterior surface rectangular, low, about ten times as wide as median height, strongly concave laterally, less so medially, smooth, without punctures; margin between dorsal and posterior surface angular, dorsal surface overlapping posterior surface laterally; scutellar pocket absent; lateral surface flat, punctured; axilla forming wide rounded ridge with small, deep punctures. (14.) Metanotum about 3 times as wide as long at middle; surface slightly concave; dorsal margin swollen, forming a rounded ridge, strongly bowed dorsad medially; ventral margin curved very slightly ventrad; surface smooth with few scattered small punctures dorsolaterally. (15.) Mesopleuron strongly convex with large, contiguous punctures dorsally, punctures about equal to size of ocellus dorsomedially, somewhat smaller ventrally, separated by one to two diameters; punctures small on anterior and posterior surfaces. (16.) Dorsal sclerite of metapleuron twice as high as wide, with few scattered small punctures; secondary suture indistinct; ventral sclerite of metapleuron smooth with scattered, very small punctures; first metapleural pit shallow; metapleural-propodeal suture not evident. (17.) Propodeum rounded, not strongly produced laterally; posterior surface with broad, shallow concavity, surface of concavity very finely rugulose without punctures; dorsolateral surface with large, contiguous, shallow punctures forming an irregular sculptured surface; lateral surface with large shallow contiguous punctures posteriorly, smaller, more scattered anteriorly; lateral ridge low, acute, extending from propodeal angle to apical scales with decreasing height; propodeal angle obtuse in lateral and caudal views, bearing small compressed lobe at apex. (18.) Thorax lightly sericeous, with very short, erect yellow hairs in punctured areas, longer on lateral areas of propodeum.

Abdomen. (19.) Tergum 1 cap-shaped, distinctly set off from tergum 2; 4.3 times as wide as long in dorsal view; sternum 1 about 5 times as wide as long; tergum irregularly rugulose, with few small punctures, sternum rugulose. (20.) Tergum 2 about .79 times as long as wide, with medium to large punctures separated by one diameter or less, smaller posteriorly, more widely spaced medially; punctures with shallow posterior furrow giving oval appearance; sternum 2 with punctures as on tergum but smaller. (21.) Terga and sterna 3-6 with dense small punctures, rugulose; tergum and sternum 6 with few small punctures. (22.) Abdomen lightly sericeous with very small, sparse, erect, yellow hairs.

Coloration. Black with yellow markings as follows: Lateral apical spots on tergum 3; apical bands on terga 4, 5; apex of tergum 6; apical and medial surfaces of sterna 3-5; sternum 6. Apex of mandible, legs, abdomen dark brown. Wings slightly infuscated along costal margin, veins dark brown.

TYPE MATERIAL. Holotype female from Corupá, Est. Santa Catarina, Brazil, A. Maller collector, in the American Museum of Natural History. Paratypes are distributed as follows: three females from Mt. Itatiaya, 700 m, Rio de Janeiro, Brazil, J. F. Zikan collector, in the Instituto Miguel Lillo, Tucumán, Argentina; two females apparently collected by Ducke bearing only the indication "Brazil, 830" and the determination labels "Nectarinia augusti var. quinta R.v.Ih.," in Collections of the Departamento de Zoologia, Secretaria de Agricultura, São Paulo, Brazil; one female, also apparently collected by Ducke bearing simply the label "830" and a determination label, "Caba (Nectarinia) augusti Sauss," in the U.S. National Museum; and one female from Guarujá, Ihla de Santo Amaro, Brazil, collected by G. E. Bryant, in the British Museum.

VARIATION. In the specimens examined, the only character that differed from the condition found in the holotype was the shape of the propodeal angle. The paratypes all had rounded propodeums in which the propodeal angle was indistinct and the lateral ridge scarcely evident.

Differences in size and proportion were also noted. The mean wing length for the sample was 6.20 mm. as opposed to 6.04 for *augusti*.

DISTRIBUTION. All specimens with the precise locality indicated were from southern Brazil including the states of Rio de Janeiro, São Paulo, and Santa Catarina. R. von Ihering's (1904) records of *Caba augusti* var. *quinta* from Minas Gerais and Paraná probably refer to *fistulosa*. The variety *quinta* was described from the upper Amazon and subsequent determinations of it, made on the basis of color alone, often extended the range erroneously.

Brachygastra augusti (Saussure)
(Figs. 35-38, 40, 41)

Nectarinia augusti Saussure, 1853-1858. Et. Fam. Vesp. p. 233 (3 ♀♀, "Capit. de Saint-Paul, Rio Grande, Boyaz," Brazil; ♀ lectotype and 2 ♀♀ syntypes in Musée National d'Histoire Naturelle, Paris, by present designation).
Nectarina augusti; Smith, 1857. Cat. Hymen. Brit. Mus. 5:137.
Nectarinia augusti var. *quinta* R. von Ihering, 1903. Ann. Soc. Ent. France 72:153 (♀♀, Est. São Paulo, Ourinno, and Rio Jurua, Amazonas, Brazil; ♀ lectotype from Rio Jurua in Dept. de Zoologia, São Paulo, Brazil, by present designation) (in part).
Nectarina augusti var. *quinta;* Dalla Torre, 1904, *in* Wytsman, Gen. Insect., fasc. 19:86.
Caba augusti; R. von Ihering, 1904. Rev. Mus. Paulista 6:106, 107.
Caba augusti var. *quinta;* R. von Ihering, 1904. Rev. Mus. Paulista 6:108, pl. 4, fig. 3.
Chartergus amazonicus Cameron, 1906. Zeitschr. Hymen. Dipt. 6:380 [♀, "Cararamer-Amazonia"; type in British Museum (Natural History)].
Nectarinia amazonica; Meade-Waldo, 1911. Ann. Mag. Nat. Hist. (8)7:111.
Brachygastra augusti; Bequaert, 1944. Bull. Mus. Comp. Zool. 94:271-272.
Brachygastra augusti augusti; Araújo, 1960. Stud. Ent. (Petropolis) 3:252.

Because of the relative stability of the color pattern of *Brachygastra augusti*, there has been little confusion as to its identity. Only one variety, var. *quinta* R. von Ihering, has been described. Unfortunately the diagnostic characters of this variety are not generally recognized, and specimens of other species have been assigned to it on the basis of color alone, although it is quite distinct morphologically.

Brachygastra augusti very closely resembles *B. lecheguana* and *B. mellifica* in color and general proportions but is considerably smaller (body length about 5 mm.) than the average sized individuals of the latter species. Occasionally specimens of *mellifica* may be as small as *augusti* but these are uncommon. *B. augusti* can be distinguished from all species of the *lecheguana* group by the rounded scutellum and the large anteriorly inflected pronotal keel (Fig. 40), and from species of the *smithii* group by the latter character and the absence of extensive yellow maculations on the head and thorax. It differs from both *azteca* and *scutellaris* by its very wide, lightly punctured, second abdominal tergum.

FEMALE. (1.) Wing length 5.82 ± .260 mm.

Head. (2.) In frontal view .88 times as high as wide; in dorsal view .42 times as long as wide; posterior margin very slightly curved. (3.) Lateral ocellus separated from eye by 1.34 times distance between lateral ocelli and from occipital carina by 1.37 times this distance; vertex with large to medium sized punctures contiguous or nearly so posterolaterally, punctures small to medium sized elsewhere, separated by one diameter or less, often contiguous; vertex slightly convex, posterior surface sloping gradually ventrad in profile. (4.) In lateral view gena about as wide as eye in middle; postgenal convexity broad, low, scarcely evident; gena about 1.3 times as wide at level of convexity as at level of eye emargination; punctures small to medium sized, separated by two diameters or less dorsally, occasionally contiguous, more scattered medially to lacking or smaller posteroventrally. (5.) Occipital carina low, acute, of even height, extending to mandibular condyle. (6.)

Frons with small to medium sized punctures, separated by about two diameters, occasionally close. (7.) Clypeus about 1.7 times as wide as long, moderately convex; distal margins curved, broadly rounded onto lateral lobes; apical triangle as long as width of antennal socket, apex broadly rounded; contact with eye equal to width of antennal socket; epistomal suture forming about 60 degree angle with eye margin, dorsally forming a broad V; surface smooth, shiny, with few fine, scattered punctures; basal 0.2 sericeous. (8.) Malar space about 0.3 width of antennal socket. (9.) Antenna with flagellum strongly swollen, eighth flagellomere twice as wide as long. (10.) Head with short, erect, gold hairs, about 0.5 to 0.8 width of ocellus in length; eye with sparse, short, erect hairs; head slightly sericeous.

Thorax. (11.) Anterior surface of pronotum smooth, with few scattered small punctures, distinctly separated from dorsal surface of pronotum; pronotal keel low, indistinct medially, absent laterally below humeral angle, developed into large, collar-like ridge at humeral angle, inflected strongly cephalad; humeral angle rounded in dorsal and lateral view; dorsal surface of pronotum evenly rounded onto narrow lateral surface, with medium to large, deep, contiguous punctures, separated by about one diameter at posterior margin; lateral surface narrow, with scattered large punctures; pronotal lobe wide, distinct. (12.) Scutum about .75 times as long as wide, with medium sized punctures, separated by three diameters or less, occasionally forming rows. (13.) In dorsal view, scutellum about three times as wide as long, moderately bilobed, about .75 as long at middle as at lateral margin, posterior margin forming a flattened V; in posterior view, about five times as wide as high at middle, dorsal margin bilobed; in profile, scutellum rounded, prominent, extending slightly over plane of metanotum; dorsal surface strongly biconvex, rounded laterally, posterior surface slightly concave; scutellar pocket absent; dorsal surface with large contiguous punctures, posterior surface smooth, dorsal punctures only slightly extended onto posterior surface; axillar ridge swollen with small contiguous punctures. (14.) Metanotum about 3 times as wide as long, slightly concave; dorsal margin bowed evenly dorsad, ventral margin slightly V-shaped; surface smooth with scattered, small, punctures. (15.) Mesopleuron very strongly convex; anterior and posterior surfaces with scattered, small punctures; large contiguous punctures dorsally, separated by one diameter or less medially, smaller, more scattered ventrally. (16.) Dorsal sclerite of metapleuron about twice as high as width at middle, with few small punctures; secondary suture indistinct; first metapleural pit deep; ventral sclerite with few scattered small punctures; metapleural-propodeal suture not evident. (17.) Propodeum angular; posterior surface with wide, shallow concavity, median area finely rugulose, without punctures; dorsolateral area with large, contiguous, deep punctures; lateral surface with very large, contiguous punctures posteriorly, smaller anteriorly, lacking ventrally; posteroventral surface rugose; lateral ridge low, acute, extending from spiracle to apical scale; propodeal angle well developed, forming a rounded right angled lobe in lateral view, an oblique shelf-like ridge in posterior view. (18.) Thorax very lightly sericeous with sparse, short, erect, gold hairs on punctured surfaces, longer on propodeum.

Abdomen. (19.) Tergum 1 flattened onto tergum 2, about 6.5 times as wide as long in dorsal view; sternum 1 little less than 6 times as wide as long; tergum with small punctures, sternum rugulose. (20.) Tergum 2 about .73 times as long as wide, high, abruptly convex in profile; tergum with dense, evenly spaced small punctures separated by about two diameters, slightly larger along lateral margin; sternum 2 with punctures more widely spaced. (21.) Terga and sterna 3-5 with dense small punctures, tergum and sternum 6 with few small punctures. (22.) Abdomen with very short erect hairs; very lightly sericeous.

Coloration. Black with yellow markings more or less developed as follows: ventral spot on inner orbit; two interantennal spots; median line on pronotal keel; dorsal margin of metanotum; apical bands on metasomal terga and sterna 2-6. Wings clear, slightly infuscated basal to stigma, veins brown.

MALE. (1.) Wing length 5.99 ± .137 mm.

As in female except for following:

Head. (2.) In frontal view .87 times as high as wide; in dorsal view .37 times as long as wide. (3.) Lateral ocellus separated from eye and from occipital carina by .85 times distance between lateral ocelli. (4.) Gena .43 times as wide as eye in lateral view; postgenal convexity absent; gena only slightly wider ventrally than dorsally. (5.) Occipital carina distinct on dorsal .7 of gena, indistinct ventrally. (7.) Clypeus .73 times as long as wide; slightly convex; distal margin almost straight, apex narrowly rounded; apical triangle about .8 times as long as width of antennal socket; contact with eye equal to about 1.3 times width of antennal socket; epistomal suture forming about a 30° angle with eye margin; clypeus entirely sericeous. (8.) Malar space very short, almost absent. (9.) Scape about 0.6 times width of clypeus; flagellum moderately swollen, eighth flagellomere about 1.9 times as wide as long.

Thorax. (11.) Pronotal keel ending abruptly at humeral angle, forming a low acute ridge across dorsal surface of pronotum, higher at humeral angle. (13.) Metanotum with dorsal surface strongly convex. (17.) Propodeum more rounded.

Abdomen. (24.) Spiculum long, narrow, about 5 times as long as basal width.

Genitalia. (25.) Paramere 2.3 times as long as high; apex truncate, angular ventrally, rounded dorsally; parameral spine about as wide at middle as at basal inflection, with dorsolateral invagination, bispinose in appearance; paramere with small, shallow notch at base of volsellar plate. (26.) Volsellar lobe 0.3 times as wide at base as long, evenly tapered to rounded apex, strongly depressed, extending to middle of digital lobe. (27.) Cuspis of volsella flattened against paramere, apex pointed in lateral view, with few, small black teeth opposite digital lobe. Digitus in lateral view thick-set, about 3 times as long as width at base, posterior angle of digital lobe forming a rounded right angle; in ventral view lobe with strong lateral swelling, posterior angle forming a rounded acute angle; lateral surface of digitus with many small short tubercules. (28.) Aedeagus in lateral view with apical lobe bent ventrad, lobe about 0.3 length of entire spatha, swollen, basally with lateral row of very small teeth; in ventral view lobe about .75 as wide at apex as at base, base about 2 times width of spatha at middle, lobe with lateral margins inflected mesad about 0.3 width of lobe, inflected margins parallel on apical 0.7 of lobe, divergent on basal 0.3; spatha gradually narrowed to point basally; ventral hook long, curved laterad; aedeagal apodeme evenly curved, small ventral swelling about 0.3 distance from base to ventral hook.

Coloration. As in female except yellow better developed, as follows: Inner orbit from eye emargination to epistomal suture; interantennal area; entire clypeus; ventral surface of scape and pedicel; anteroventral surfaces of coxae, trochanters, fore and mid femora. Flagellum ferruginous ventrally.

Type Material. There are three specimens of *Brachygastra augusti* labeled "TYPE" in the Paris Museum. They have the following locality labels: "Museum Paris, Sud de la Capit.^{e} de Goyaz"; "Museum Paris, Bresil, Rio Grande, G. St. Hilaire"; "Museum Paris, Nord Capit.^{e} de St. Paul, St. Hilaire." Saussure's locality, "Capit. de Saint-Paul, Rio Grande, Goyaz, Bresil", then, cannot refer to any one of the specimens. Capit.^{e} (= Capitainerie) probably refers to the state or captaincy at that time and does not represent a precise locality. I have designated the specimen with the Rio Grande label as lectotype because it is in the best condition. It bears a second circular label reading "Capit.^{e} de Rio Grande." It agrees closely with specimens from Goias and with the foregoing description.

R. von Ihering's variety *quinta* was described from a series of specimens in the Museo Paulista but only four specimens (3 ♀♀, 1 ♂) there now can be definitely said to be of his type series. These are from the Rio Jurua, Amazonas, Brazil, 1902, E. Garbe collector. I am designating one of these, a female, as lectotype. Other specimens in the museum labeled "Franca, S. P., Brazil, 1902, O. Dreher collector, could possibly be the São Paulo specimens which von Ihering mentioned. Three additional specimens labeled Brazil, 830, which I have included in the type series of *B. fistulosa* n. sp., could also have been in his type series.

I have seen a specimen of *B. augusti* from Misiones, Argentina, which has been compared with the type of *Chartergus amazonicus* Cameron, and it leaves no doubt as to the synonymy of the latter species.

Distribution. *B. augusti* extends from Costa Rica to southern Brazil. It is common throughout the Amazon basin but is not found in the drier areas of northeastern Brazil and the southern Guianas. In Perú and Colombia it extends into the foothills of the Andes and has been collected as high as 1500 m. The southernmost locality that I have seen is in Santa Catarina, Brazil, but Ducke (1910) records it from Rio Grande do Sul. It occurs in eastern Paraguay but has been collected only in the Province of Misiones in Argentina.

Specimens have been examined from the following localities. CENTRAL AMERICA. *Costa Rica.* Cartago Prov.: Turrialba. Heredia Prov.: Puerto Viejo. Limón Prov.: Limón. (Colima; Colimbiana Farm, Santa Clara Prov.). *Panamá.* Canal Zone: Ancón; Balboa; Corozal; Juan Mina Station and Plantation; Panamá; Punta Paitilla; Quebrada Bonita; Red Tank; Sabanas. Colón Prov.: Portobelo. (Tabernilla).

SOUTH AMERICA. *Argentina.* Prov. Misiones: (Bemberg); Iguazú. *Bolivia.* Dept. Beni: Riberalta; Rurrenabaque, 175 m; Santa Elena; Trinidad, Río Pan. Dept. La Paz: Corioco; Yungas. Dept. Pando: Río Mapirí. Dept. Santa Cruz: Santa Cruz, 500 m. *Brazil.* Est. Acre: Iquiri. Est. Amazonas: Manaus; Obidos; (Pevas); Rio Jurua. Est. Goiás: 24 km. E. Formoso; Jatai, Faz. Cachoeirinha. Est. Mato Grosso: Chapada; (Itapura); Porto Velho, Cicade Branco; (Rio Caraguatá); Utiariti, Rio Papagaio; West Border. Est. Pará: (Anamindena); Santarem; Lower Rio Liberdade. Est. Paraná: Caviuna. Est. São Paulo: Franca; Guarulhos; Itaituba; Itápolis; (Lussavira); Porto Cabral; São Paulo; Santo Amaro Island. *British Guiana.* Berbice Co.: New River, 750 ft. Essequibo Co.: Mt. Roraima; Rio Essequibo, Source. *Colombia.* Dept. Boyaca: Porto Olaya, 100 m; Restrepo, 500 m. Dept. Caldas: 12 m S. Anserma, 1030 m. Dept. Cauca: (Hormiguero); Cauca Valley, 3260 ft.; (Hacienda García); Cauca Valley. Dept. Santander: Landazuri, 500 m; San Vicente de Chucurí. Dept. Tolima: Coyaima, 450 m. Dept. Valle: Cali district; Cauca Valley, 3260 ft.; 40 mi. S. Cali, 1140 m. *Ecuador.* Napo-Pastasa Prov.: Puyo, 2000-2500 ft. *French Guiana.* Dept. Guyane: St. Jean du Maroni. *Paraguay.* Dept. Guaira: Colonia Independencia. *Perú.* Dept. Cajamarca: Jaén. Dept. Huánuco: Tingo María; Monzon Valley; Rio Huallaga, 670 m; Yurac, 67 m. E. Tingo María. Dept. Loreto: Divisória; Cordillera Azúl, 1300 m; Iquitos; (Río Charape); (Río Cotuhe); Río Putumayo, near Taona. Dept. Pasco: Iscozazín. Dept. San Martín: San Martín, 1500 ft. *Surinam.* Dist. Saramacca: Kwakoegron. Dist. Suriname: Paramaribo; Republiek; (Auca on Suriname River). *Venezuela.* Est. Bolivar: Parai-Tepuí. Est. Carabobo: Puerto Cabello; San Esteban. Est. Yaracuy: Laguna de Aroa, 2000 ft.

Variation. In order to gain a thorough understanding of the variation in this species, a large number of males should be examined. The females vary little throughout the range, differing only in punctations and slight differences in the scutellum and propodeum. These differences are continuous at any one locality and cannot be considered geographical variation. R. von Ihering's variety is based on a number of specimens from the Rio Jurua which are heavily punctured, but this condition is found also in São Paulo and Venezuela.

In northern Venezuela there is an interesting variation which differs strikingly from the condition found in all other specimens of *augusti* studied. This is the development of the ocular swelling into a distinct cone-shaped prominence. Wasps of this type also have smaller punctures on the dorsal surface of the pronotum and somewhat larger punctures on the abdomen. Half of the specimens studied from Venezuela were of this type and occurred together with the typical form. Such specimens may represent a distinct population in Venezuela which should be considered a separate species, but until additional material including males becomes available, I do not feel justified in separating it.

Only four males of *augusti* were available for the present study but these were of two distinct types. Two from Jaén, Dept. Cajamarca, Perú, and Trinidad, Dept. Beni, Bolivia, had elongate volsellar lobes and angular digital lobes, and two from Rio Jurua, Est. Amazonas, Brazil, and Muzo, Dept. Boyaca, Colombia, had the shorter tapered volsellar lobes and the rounded digital lobes as described above. It appears then, that there may be two sibling species separable only on the basis of male characters. These species would be broadly sympatric as the males of both are found in widely separated areas in the western Amazon basin. Here again, only the study of males from other areas throughout the range of *augusti* could clarify the situation and facilitate their separation.

The color of this species varies primarily with respect to the width of the abdominal bands. In Costa Rica and Panamá these are very reduced dorsally and present only on the apical segments. In South America the width of the bands varies throughout the range and is only rarely as wide as in *lecheguana*.

Biology. The nest of this species has been described by R. von Ihering (1904), Ducke (1910) and Bertoni (1911). It is very similar to small nests of the *Polybia occidentalis* group but is more ovoid and fragile. R. von Ihering described the entrance hole as being a vertical slit, 5 × 30-40 mm. as in *Polybia singularis* Ducke. The nest he described had 7 combs and was 10 cm. high and 13 cm. maximum diameter.

I have seen a small ovoid nest in Costa Rica which had been recently founded by 780 wasps. The nest was 6 cm. high and 8 cm. at its greatest diameter with only 4 combs of shallow, incomplete cells. Oviposition had not begun and the main activity was that of enlarging the nest. It appears that the vertical entrance hole is formed by the excentric passageways between the combs being placed one above the other and all opening to the outside. This is unlike many other phragmocyttarous nests which have only one opening, that of the bottom comb which then serves as an entrance to the entire nest. With the former type, all combs open directly to the outside.

In Costa Rica *B. augusti* is found in the cultivated, wet lowlands where I have seen it visiting flowers of *Lantana* and *Hyptis obtusiflora*. I have not seen it in the dry lowlands or at higher altitudes but it has been collected as high as 1500 m. in the Andes.

The *B. smithii* Group

The species of this group have the following characteristics: occipital carina well developed on ventral half of gena; postgenal convexity present, more or less well developed; pronotal keel prominent, projecting cephalad on humeral angle; scutellum rounded; yellow color pattern variable, often extensive and including maculations on the vertex, gena, scutum, scutellum, metanotum, propodeum, and the discal area of the second abdominal tergum. The nests of this group that have been described are small, ovoid and relatively short lived.

Due to the extensive variation of the color pattern of the species in this group, many species and varieties have been described and treatment of these forms varies throughout the literature. The species recognized here are as follows: *B. baccalaurea* (R. von Ihering) in northern Andean regions; *B. bilineolata* Spinola, Venezuela to southern Brazil; *B. smithii* (Saussure), southern Mexico to southern Brazil; *B. propodealis* Bequaert, southwestern Amazon Basin; *B. buyssoni* (Ducke), central Amazon Basin.

Brachygastra baccalaurea (R. von Ihering) new combination
(Figs. 61, 65)

Nectarinia baccalaurea; R. von Ihering, 1903. Ann. Soc. Ent. France 72:154-155 (no sex or locality given; ♀ lectotype from Perú in Departamento de Zoologia, São Paulo, Brazil).
Nectarinia baccalaurea; Dalla Torre, 1904. *in* Wytsman, Gen. Insect., fasc. 19:86.
Caba baccalaurea; R. von Ihering, 1904. Rev. Mus. Paulista 4:106, pl. 4, fig. 4.

This extraordinary species, described from Perú, has the greatly developed pubescence that is often characteristic of Hymenoptera from Chile and the Andean regions of western South America. Von Ihering's description correlates perfectly with the one specimen found in the collection at the Departamento de Zoologia, São Paulo.

Both in size and general proportions this species very closely resembles *lecheguana*, as von Ihering pointed out. *B. baccalaurea* is easily distinguished from the latter, however, by its characteristic rounded scutellum and the unusual form of the metanotum which bears a median dorsal projection (Fig. 65). A closer comparison shows the abdomen of *lecheguana* to be relatively wider than *baccalaurea*. Two additional characters, the black wings and the yellow markings, also separate the latter from *lecheguana* but not from other species of the *smithii* group. The long, gold hairs which are abundant on the head and thorax distinguish it from other species with similar coloration.

FEMALE. (1.) Wing length 8.10 ± .340 mm.

Head. (2.) In frontal view .85 times as high as wide; in dorsal view .37 times as long as wide; posterior margin strongly curved. (3.) Lateral ocellus separated from eye by 1.6 distance between lateral ocelli and from occipital carina by 1.2 times this distance; vertex with small punctures separated by one diameter or less; vertex strongly convex, sloping ventrad in profile. (4.) Gena about 1.25 times as wide as eye in lateral view; gena with broad, medium sized postgenal convexity, about 0.5 length of entire gena; gena about 1.3 times as wide at level of convexity as at level of eye emargination; punctures small to medium sized, separated by about three diameters dorsally, slightly smaller and more scattered ventrally, very small or lacking on postgenal convexity. (5.) Occipital carina low, distinct, rounded medially, obscure and irregular on temporal convexity, becoming a high, curved, blade-like ridge on postgenal convexity ending at mandibular condyle. (6.) Frons with dense small punctures separated by one diameter or less. (7.) Clypeus about 1.6 times as wide as long, moderately convex; distal margin straight, narrowly rounded onto lateral lobe; apical triangle about as long as width of antennal socket, apex narrowly rounded; contact with eye equal to 0.5 width of antennal socket; epistomal suture forming about a 60° angle with eye margin, dorsally evenly rounded; clypeal surface smooth, shiny with few scattered fine punctures, basal 0.3 sericeous. (8.) Malar space about 0.8 width of antennal socket. (9.) Antenna with flagellum moderately swollen, eighth flagellomere about 1.6 times as wide as long. (10.) Head with abundant, long, golden hairs recurved distally, lengths equal to three times width of ocellus on vertex and frons, equal to little more than width of ocellus elsewhere; eyes densely pubescent; head densely sericeous.

Thorax. (11.) Anterior surface of pronotum with scattered small to medium sized punctures dorsolaterally, in profile distinctly separated from dorsal and lateral surfaces by pronotal keel; pronotal keel low and rounded medially, forming a low acute ridge dorsolaterally, low or absent immediately below humeral angle, forming acute blade-like ridge ventrolaterally; humeral angle more or less acute, forming a point projecting cephalad; dorsal surface sloping strongly onto lateral surface forming one oblique dorsolateral surface, with shallow, medium to large punctures contiguous or nearly so; posterior pronotal lobe wide, distinct. (12.) Scutum about .82 times as long as wide, evenly covered with small to medium sized punctures separated by two diameters or less. (13.) In dorsal view scutellum about twice as wide as long, low and evenly rounded in all aspects; dorsal surface only slightly flattened, not distinct from posterior surface; scutellar pocket absent; punctures medium sized, contiguous, smaller and not contiguous along posterior margin. (14.) Metanotum about 3.3 times as wide as long, convex; dorsal margin with a large medial projection extending over posterior margin of scutellum in caudal view; punctures small, scattered dorsally. (15.) Mesopleuron strongly convex; anterior and posterior surfaces with scattered small punctures; punctures medium sized medially, separated by one diameter or less, surface rugose dorsomedially. (16.) Dorsal sclerite of metapleuron 2.5 times as high as wide at middle, with scattered small punctures dorsally; secondary suture distinct; first metapleural pit deep with broad, shallow concavity; ventral sclerite with few small punctures ventrally; metapleural-propodeal suture evident as weak furrow. (17.) Propodeum rounded; posterior surface with wide, shallow concavity, median area rugulose, dorsolateral areas rugose, punctures indistinct; lateral surface, rugose, irregularly sculptured posteriorly, with medium to small, contiguous punctures anteriorly; lateral ridge acute, extending from spiracle to apical scale, decreasing in height gradually posteriorly; propodeal angle bearing an obtuse, rounded, flattened, lobe in profile, a rounded ridge in caudal view. (18.) Thorax with long, golden hairs, recurved distally on punctured and sculptured surfaces; thorax heavily sericeous.

Abdomen. (19.) Tergum 1 convex, distinct from tergum 2, about 4 times as wide as long in dorsal view; sternum 1 about 4 times as wide as length at middle; tergum with scattered small punctures, sternum rugulose. (20.) Tergum 2 about .85 times as long as wide; depressed, low, evenly convex in profile; tergum and sternum 2 with small punctures separated by three diameters or less. (21.) Terga 3-6 and sterna 3-5 rugose with small apical punctures; sternum 6 with scattered small punctures. (22.) Abdomen with long, golden hairs, lightly sericeous.

Coloration. Black with yellow markings more or less developed as follows: inner orbit from epistomal suture to eye emargination; oblique line lateral to ocelli forming flattened V pointing posteriorly; dorsal portion of pronotal keel; two parallel, longitudinal scutal lines; axilla; anterior margin of scutellum; dorsal margin of metanotum; apical bands on terga 1-6 and sterna 1-5, discal band on tergum 2 with lateral or posterior emarginations. Wings infuscated with very dark brown, veins black.

MALE. Not seen. The following characters are extracted from Buysson's (1905) description and figure.

Genitalia. (24.) Apex of paramere blunt, not truncate; parameral spine very wide at base. (25.) Volsellar lobe long, extending beyond middle of digitus, wide, with long tactile hairs mesally. (26.) Digitus thick-set, about as wide at base as distance between apex and ventral angle; in lateral view posterior angle rounded, blunt, ventral angle acute; cuspis rounded apically. (27.) Aedeagus with ventral hook well developed.

Coloration. As in female except yellow markings developed as follows: ventral surface of scape; four spots in place of V on vertex; yellow lacking on scutellum. There is no reason to expect the male to be any less variable than the female. Further collections should include males with color patterns as well developed as or even more extensive than those observed in the female.

TYPE MATERIAL. The type collected by R. von Ihering in 1902 is in the collection of the Departamento de Zoologia, Secretaria de Agricultura, São

Paulo, Brazil (Museu Paulista). It bears no indication other than "Peru 827." There is a small circular blue label followed by a determination label, "Nectarinia baccalaurea, R. v. Ih. Ducke rev. 11." It is in good condition. As this is the only specimen in the Museum, I have placed a lectotype label on it. According to Ducke (1910), there is an additional cotype in the Paris Museum but this has not been found.

VARIATION. Even though the number of specimens examined was small, the variation both in morphology and coloration was considerable. Von Ihering's type from Perú has fairly well developed humeral and propodeal angles but this condition is moderate when compared with the one specimen from Bolivia in which these angles, especially the latter, approach the extreme condition found in *buyssoni*. The difference between these two specimens is so striking that it may well warrant the recognition of the Bolivian population as a distinct species but until further material is examined, I do not feel that separation is justified.

Other specimens examined from Perú displayed slight variation in the development of the humeral angle but this was always a reduction of the condition found in the type.

The majority of wasps examined were melanic forms, the yellow being present only as spots on the vertex and metanotum and the usual apical bands on the abdominal segments. A few specimens from Ecuador and Colombia showed a gradation from largely melanic to extensive yellow coloration resembling in pattern that found in other species of the *smithii* group. One wasp from Colombia (Caldas Salento) had very extensive yellow markings including the distal band on the second abdominal segment. Although it was smaller, I could find no substantial morphological differences between it and the Peruvian specimens. I expect that additional collections will show this species to be as variable in color pattern as other species of the *smithii* group, and description of specific and subspecific categories should be done with discretion.

DISTRIBUTION. *B. baccalaurea* seems to be restricted to the uplands of Colombia, Ecuador, Perú and Bolivia. The specimens I have seen are all from altitudes of 1600-1900 meters. The greatly developed pubescence and long wings also suggest that it is a species of high altitudinal ranges.

Specimens have been examined from the following localities: *Bolivia.* Dept. La Paz: Mapiri. *Colombia.* Dept. Boyaca: Caldas Salento, 1900 m. *Ecuador.* Prov. Napo-Pastaza: Baeza. Prov. Tungurahua: Baños, 1600-1900 m. *Perú.* Dept. Junin: Huacapistana on Río Tarma, 1800 m.

Brachygastra bilineolata Spinola

(Figs. 51-56, 59, 68)

Brachygastra bilineolata Spinola, 1841. Ann. Soc. Ent. France 10:126 (2 ♀♀, Cayenne, French Guiana; in the Museo di Zoologia, Torino).

Brachygastra dorso-lineata; Spinola, 1841. Ann. Ent. Soc. France 10:123. Clerical error for *Brachygastra bilineolata* Spinola, 1841.

Nectarinia bilineolata; Saussure, 1853-1858. Et. Fam. Vesp. 2:226, 228, 231, pl. 34, fig. 2.
Nectarinia bilineata; Saussure, 1853-1858. Et. Fam. Vesp. 2:231. Misspelling of *bilineolata.*
Nectarina bilineolata; Smith, 1857. Cat. Hymen. Brit. Mus. 5:136.
Nectarinia möbiana Saussure, 1867. Reise der Novara, Zool. 2(1):22 (6 ♀♀ from Surinam).
Nectarina möbiana; Dalla Torre, 1904. *in* Wytsman, Gen. Insect., fasc. 18:86.
Odynerus antillarum Provancher, 1888. Additions Faune Canada, Hymen., p. 420 (♀, Trinidad).
Caba moebiana; R. von Ihering, 1904. Rev. Mus. Paulista 4:106.
Caba bilineolata; R. von Ihering, 1904. Rev. Mus. Paulista 4:106, 111.
Caba bilineolata var. *fasciata* R. von Ihering, 1904. Rev. Mus. Paulista 4:112 (no sex given, Surinam, Rio Juruá, Amazonas, Brazil).
Nectarina bilineolata var. *moebiana;* Buysson, 1905. Ann. Soc. Ent. France 74:547, 552.
Nectarina bilineolata var. *fasciata;* Ducke, 1905. Bol. Mus. Goeldi 4:663.
Nectarina bilineolata moebiana; Ducke, 1918. Rev. Mus. Paulista 10:327.
Nectarina bilineolata var. *smithi;* Vesey-Fitzgerald, 1938. Trans. Roy. Ent. Soc., London 87:184 (misidentification).
Brachygastra bilineolata var. *antillarum;* Bequaert, 1942. Jour. New York Ent. Soc. 50:304.
Brachygastra bilineolata var. *smithii;* Bequaert, 1942. Jour. New York Ent. Soc. 50:305.
Brachygastra bilineolata var. *surinamensis* Bequaert, 1942. Jour. New York Ent. Soc. 50:306 (♀ holotype, Surinam; in the Museum of Comparative Zoology, Harvard).

Spinola's original description unfortunately was concerned primarily with pubescence and coloration, and his species was consequently restricted to forms with similar color patterns whether or not they differed morphologically. *B. smithii* was described as a separate species by Saussure and was considered as such by Buysson (1905), but Ducke (1907) placed it as a variety of *bilineolata.* Bequaert (1942) added *Odynerus antillarum* Provancher and two new varieties, *surinamensis* and *propodealis*, to the list of varieties of *bilineolata.* This study separates *smithii* and *propodealis* as species distinct on a morphological basis and having a color variation parallel to that of *bilineolata.* References to any of the above forms, therefore, could refer to any one of the three distinct species.

B. bilineolata is about 6 mm. long (h + th + terg 2) and is variously marked with brilliant yellow. In general appearance it is often identical with *smithii* but can be separated by examination of the following characters. In northern South America it is separable on the basis of the occipital carina which is low or absent on the ventral half of the gena. *B. smithii* in the same region has a well developed carina. In the southern part of its range, *i.e.*, south of the Amazon, the occipital carina becomes very high but that of *smithii* is, in contrast, much reduced. This character, then, is inversely related in these two species. The scutellum of *bilineolata* is lower than that of *smithii* and does not project strongly dorsad. *B. bilineolata* also has relatively fewer punctures, giving the cuticle a shiny appearance not found in *smithii.* This latter character will serve to distinguish it from *propodealis* in Bolivia and western Amazonas where all three species are found together.

FEMALE. (1.) Wing length 5.98 ± .302 mm.

Head. (2.) In frontal view .87 times as high as wide; in dorsal view .42 times as long as wide; posterior margin slightly curved, almost straight. (3.) Lateral ocellus separated from eye by 1.24 times distance between lateral ocelli and from occipital carina by 1.37 times this distance; vertex with small to medium sized punctures separated by about one diameter; vertex slightly convex, posterior surface sloping slightly ventrad in profile. (4.) In lateral view gena about as wide as width of eye at middle; postgenal convexity small, irregularly rounded, ventral margin of convexity straight; gena about 1.4 times as wide at level of convexity as at level of eye; punctures medium sized, separated by one diameter or less dorsally, more scattered ventrally, small or absent on convexity and along ventral margin. (5.) Occipital carina low, acute, of even height, extending almost to mandibular condyle, indistinct immediately posterior to condyle. (6.) Frons with small to medium sized punctures separated by about one diameter. (7.) Clypeus about 1.6 times as wide as long; moderately convex; distal margin straight, broadly rounded onto lateral lobe; apical triangle long, about equal to width of antennal socket, apex broadly rounded; contact with eye equal to about 0.5 times width of antennal socket; epistomal suture forming about a 70° angle with margin of eye, dorsally broadly curved; clypeal surface smooth, shiny, with few small punctures, basal 0.3 sericeous. (8.) Malar space 0.5 width of antennal socket. (9.) Antenna with flagellum moderately swollen, eighth flagellomere 1.7 times as wide as long. (10.) Head with abundant, long, golden hairs, about as long as width of ocellus on vertex, shorter elsewhere; eyes with abundant short hairs, about 0.3 width of ocellus in length; head lightly sericeous.

Thorax. (11.) Anterior surface of pronotum with few scattered punctures, distinctly separated from dorsal surface, forming an obtuse angle with dorsal surface in profile; pronotal keel low, acute, extending almost to ventral extremity of pronotum, only slightly higher at humeral angle than elsewhere; humeral angle not well developed, rounded; dorsal surface evenly curved onto lateral surface, swollen posteriorly, with deep, medium sized punctures, contiguous or nearly so; lateral surface narrow, distinct from anterior surface, with medium sized punctures; pronotal lobe wide, distinct. (12.) Scutum about .75 times as long as wide with large deep punctures separated by about one diameter. (13.) Scutellum rounded; in dorsal view about 3 times as wide as long at middle, moderately bilobed, about 0.8 times as long at middle as at lateral margins, lateral margins rounded, posterior margin curved; in posterior view 4 times as wide as high at middle, lateral margins rounded, dorsal margin strongly indented medially; in profile scutellum rounded, prominent, projecting slightly over plane of metanotum; dorsal surface convex, posterior surface concave; scutellar pocket flat; dorsal surface of scutellum rounded onto lateral surface; scutellar pocket punctured posteriorly; dorsal surface with large, deep contiguous punctures, posterior surface smooth with few punctures dorsally; axillar ridge broadly rounded with small punctures. (14.) Metanotum about 3 times as wide as long, slightly concave; dorsal margin bowed slightly dorsad, ventral margin bowed slightly ventrad; surface smooth with few fine punctures dorsally. (15.) Mesopleuron strongly convex; anterior and posterior surfaces with scattered, small punctures; punctures medium to large sized medially, separated by about one diameter. (16.) Dorsal sclerite of metapleuron about 2.3 times as high as width at middle with scattered small punctures; secondary suture indistinct; first metapleural pit more or less wide, deep; ventral sclerite with few scattered small punctures; metapleural-propodeal suture scarcely evident, forming a weak furrow. (17.) Propodeum moderately angular; posterior surface with broad, deep, central concavity, median area smooth, dorsolateral area with contiguous, medium sized, deep punctures; lateral surface with contiguous medium to large sized punctures posteriorly, smaller and scattered anteriorly, surface rugose ventrally; lateral ridge low, irregular, extending to apical scales, considerably higher on propodeal angle; propodeal angle well developed, appearing as a rounded obtuse angle laterally, a rounded lateral extension posteriorly. (18.) Thorax with abundant, short, golden hairs on punctured surfaces; lightly sericeous.

Abdomen. (19.) Tergum 1 moderately flattened onto tergum 2, about 5.2 times as long as wide in dorsal view; sternum 1 about 4 times as wide as length at middle; tergum with few fine punctures, sternum rugose with sharp transverse median ridge. (20.) Tergum 2 about .73 times as long as wide, high, abruptly curved in profile; surface shiny with small punctures separated by about two diameters, punctures larger on sternum. (21.) Terga and sterna 3-5 with punctures as on 2; tergum and sternum 6 with few or without punctures. (22.) Abdomen with sparse, short, white hairs directed caudad, lightly sericeous.

Coloration. Very variable (Fig. 68). Black with yellow markings more or less developed as follows: lateral and distal margins of clypeus; inner orbits; interantennal area; two oblique bars and a median anterior spot on vertex; anterior half of gena; dorsal surface of pronotum; tegula; two longitudinal bands joined posteriorly on scutum; scutellum; metanotum; subtegular spot on mesopleuron; apical bands on terga 1-5 and sterna 2-5; tergum 2 with or without median discal band or entirely yellow; tergum and sternum 6. Apex of mandible, flagellum and legs dark brown. Wings yellow, darkened apically.

MALE. (1.) Wing length 5.8 mm.

As in female except for following:

Head. (2.) In frontal view .86 times as high as wide; in dorsal view .39 times as long as wide. (3.) Lateral ocellus separated from eye by about .84 times distance between lateral ocelli and from occipital carina by .88 times this distance; vertex strongly convex, posterior surface sloping strongly ventrad in profile. (4.) In lateral view gena .42 times as wide as eye at middle; postgenal convexity absent; gena narrowed dorsally, about 1.5 times as wide at level of convexity as at level of eye emargination. (5.) Occipital carina low, rounded dorsally, somewhat higher and acute laterally, absent on ventral third of gena. (7.) Clypeus .72 times as long as wide, slightly convex; distal margin curved; apical triangle about as long as width of antennal socket, apex broadly rounded; contact with eye equal to about 1.3 times width of antennal socket; epistomal suture forming about a 30° angle with eye margin; clypeus entirely sericeous. (9.) Antenna with scape about 0.5 times as long as width of clypeus; flagellum moderately swollen, eighth flagellomere 1.5 times as wide as long.

Thorax. (11.) Anterior surface of pronotum evenly curved onto dorsal surface, not distinctly separated from dorsal surface; pronotal keel very low, rounded, indistinct; humeral angle absent; dorsal surface evenly curved onto lateral surface; lateral surface somewhat distinct from anterior surface, with low rounded ridge. (13.) Scutellum strongly convex dorsally; margins rounded; posterior surface with large punctures. (12.) Propodeal angle broadly rounded in lateral view.

Abdomen. (24.) Spiculum long, narrow, about 5.3 times as long as width at base.

Genitalia. (25.) Paramere about 2.1 times as high as wide; apex roundly truncate; parameral spine about 0.8 times as wide at middle as at basal inflection, with slight lateral invagination; without notch at base of volsellar plate. (26.) Volsellar lobe long, wide; in lateral view about 3 times as long as width at base; in ventral view extending little beyond middle of digital lobe, evenly tapered to rounded apex; lobe strongly flattened. (27.) Cuspis appressed against paramere, apex rounded; bearing few teeth opposite base of digitus. In lateral view, digitus thick-set rounded; about 2.3 times as long as width at base, posterior angle broadly rounded, almost reaching apex of aedeagus, ventral angle broadly rounded, directed anteroventrally; in ventral view digital lobe ovoid, greatly widened basally; digitus with small black tubercles laterally. (28.) Aedeagus in lateral view curved ventrad, aedeagal lobe about 0.2 length of entire spatha, lobe strongly swollen; in ventral view lobe rounded, about 1.3 times as wide as width of spatha at middle, inflected margins meeting at apical fourth of lobe, gradually divergent basally; spatha abruptly expanded at base of lobe to about 2 times width of spatha at middle, expansion with row of fine teeth along lateral margin; basal third of spatha with lateral margins gradually convergent to base; ventral hook long, curved laterad apically; aedeagal apodeme angular, widened apically.

Coloration. As in female except for following: yellow markings more extensive as follows: entire surface of clypeus; ventral surface of scape and flagellum; anteroventral surfaces of coxae 1 and 2 and trochanter 1. Flagellum light brown dorsally.

TYPE MATERIAL. Spinola's two female types are in the Museo di Zoologia, Torino. They bear the following labels "Brachygastra bilineolata, m., Ann. Soc. Ent., Cayenne, D. Buquet, M. Leprieur." One of these has lost its head but otherwise they are in good condition. The female with a head is designated lectotype. They both agree closely with the description given above.

I have not been able to locate Saussure's types of *Nectarinia moebiana*, but Buysson indicates that they are in the Natural History Museum at Geneva. There are five specimens labeled *moebiana* in Saussure's collection in Geneva but these do not bear type labels and two are without any labels.

I have also not been able to locate von Ihering's type of var. *fasciata*, and its listing as a synonym of *bilineolata* is on the basis of his original designation. It is quite possible that his types are *smithii* because a specimen determined by him in 1911 as *bilineolata* var. *fasciata* is *smithii.*

VARIATION. The morphological variation present in this species suggests that it may be divisible into two species or at least two subspecies. Specimens from south of the Amazon differ from the above description in the following manner: the occipital carina is high on the postgenal convexity; the scutum has medium sized punctures relatively widely spaced; the scutellum is not as high and is distinctly bilobed, the posterior surface being concave medially; the color variation is not as wide, the scutal lines and discal band being only rarely present. This latter difference is particularly interesting because north of the Amazon the loss of the discal band is not always associated with loss of the scutal lines.

Specimens from Peru and Bolivia agree with those from southern Brazil and specimens from Colombia, Venezuela and Trinidad are like those of the Guianas.

The color variation in *bilineolata*, as noted above, is most striking in the northern part of its range. In Surinam, wasps from the savannas have the entire dorsal surface of the second abdominal tergum yellow (= var. *surinamensis* Bequaert) (d, Fig. 68), but other specimens from northern Surinam often have only the apical band (a, Fig. 68). There does not appear to be a predominance of any one form as many forms are common throughout the range, but at any one specific locality the variation is slight. In Trinidad, for example, all specimens seen had a wide discal band and well developed scutal lines but in the Orinoco delta, south of Trinidad, specimens are very dark with the discal band and scutal lines absent or incomplete.

DISTRIBUTION. *Brachygastra bilineolata*, as treated here, does not extend into Central America but is widespread throughout South America being sympatric with the closely related *smithii.* Ducke (1910) states that it is found only in dry forests and savannas. The extremely yellow specimens (= var. *surinamensis*) from the savannas of the Guianas indicate that it is found in these drier areas but the color variation throughout the range suggests that it is not restricted to this habitat. In Peru it has been collected as high as 1200 m. (San Ignacio).

Specimens have been examined from the following localities: *Bolivia.* Dept. Beni: Cavinas. Dept. La Paz: Covendo; (Prov. del Sara, 450 m). *Brazil.* Est. Goiás: Jatai. Est. Guaporé: Pôrto Velho. Est. Mato Grosso: Chapada; (Rio Caraguata). Est. São Paulo: (Eng. Cesar de Souza); (Ilha Seca). Est. Santa Catarina. *British Guiana.* Berbic Co.: Ituni savanna. Demerara Co.: Georgetown; Wismar. Essequibo Co.: Rupununi savanna. *Colombia.* Dept. Meta: Villavicencio. *French Guiana.* Dept. Guyane: Cayenne. *Perú.* Dept. Cajamarca: San Ignacio, 1200 m. Dept. Loreto: Dos de Mayo, El Porvenir. Dept. Pasco: Cam. del Pichis; (Valle Chanchamayo; Río Pampaconas). *Surinam.* Dist. Commewijne: Alliance; Marienburg. Dist. Marowijne: Albina. Dist. Suriname: Blauwgrond; Clevia; Paramaribo; Zanderij savanna. *Trinidad.* Mayaro Bay. *Venezuela.* Est. Anzoategui: Guanta. Est. Bolívar: Ciudad Bolívar. Terr. Delta Amacuro, 140 kms. N.E. Barrancas.

Brachygastra smithii (Saussure)

(Figs. 46-49, 57, 58, 60, 62-64, 67)

Nectarinia smithii Saussure, 1853-1858. Et. Fam. Vesp. 2:229, pl. 31, fig. 8 (♀, Santarém, Brazil).
Nectarina smithii; Smith, 1857. Cat. Hymen. Brit. Mus. 5:136, pl. 5, fig. 2.
Caba smithi; R. von Ihering, 1904. Rev. Mus. Paulista 4:106, 112-113.
Nectarina smithi; Dalla Torre, 1904. *in* Wytsman, Gen. Insect., fasc. 18:86.
Nectarina bilineolata; Ducke, 1904. Bol. Mus. Goeldi 4:322 (in part).
Nectarina bilineolata var. *smithi;* Ducke, 1907. Bol. Mus. Goeldi 5:156, 157.
Nectarina bilineolata smithi; Ducke, 1918. Rev. Mus. Paulista 10:327.
Nectarina bilineolata var. *smithii;* Dover, 1924. Psyche 31(6):307 (in part).
Brachygastra bilineolata var. *smithii;* Bequaert, 1942. Jour. New York Ent. Soc. 50:305 (in part).

Although the type of this species has not been seen, Saussure's description, "Ecusson surplombant, bituberculé, tres grossièrement ponctué," applies well to this species. His description of the color pattern, however, has caused all forms with a median transverse band on the second abdominal tergum to be placed in this species without consideration of additional characters. Ducke (1907) placed *smithii* as a variety of *bilineolata* and it has not been considered a species since then. Examination of morphological characters has shown it to be a distinct species with variation in color pattern paralleling that of *bilineolata*.

It can be distinguished from *bilineolata* in northern South America by the high, well developed occipital carina (Fig. 47) and the projecting, bilobed scutellum (Fig. 57). South of the Guianas, however, the carina becomes reduced, but the scutellum remains diagnostic. In the eastern Amazon region it occurs together with *propodealis* but is distinct on the basis of the heavy punctures on the scutum, the reduced occipital carina, and the prominent scutellum.

FEMALE. (1.) Wing length 6.31 ± .332 mm.

Head. (2.) In frontal view .88 times as high as wide; in dorsal view .40 times as long as wide; posterior margin slightly curved. (3.) Lateral ocellus separated from eye by 1.33 times distance between lateral ocelli and from occipital carina by 1.36 times this distance; vertex with large punctures separated by one diameter or less, often contiguous behind eye; vertex slightly convex, sloping ventrad along posterior margin only. (4.) In lateral view gena about 1.1 times as wide as eye at middle; postgenal convexity moderately developed, broadly rounded; gena about 1.6 times as wide at level of convexity as at level of eye emargination; punctures large, separated by about one diameter or less dorsally, small and more scattered ventrally, very small and sparse on convexity. (5.) Occipital carina low, acute dorsally, forming an acute, narrow ridge laterally, high, blade-like, equal to about .3 width of ocellus on convexity. (6.) Frons with medium to large sized punctures separated by one to two diameters. (7.) Clypeus about 1.7 times as wide as long, moderately convex; distal margin straight, narrowly rounded onto lateral lobe; apical triangle long, little longer than width of antennal socket, apex narrowly rounded; contact with eye equal to about .7 width of antennal socket; epistomal suture forming about a 70° angle with margin of eye, dorsally forming a flattened V; clypeal surface smooth, with few small punctures, basal 0.3 sericeous, distal marginal hairs sparse. (8.) Malar space 0.5 width of antennal socket. (9.) Antenna with flagellum moderately swollen, eighth flagellomere 1.8 times as wide as long. (10.) Head with long, golden, hairs, more or less abundant, about as long as width of ocellus on vertex, shorter elsewhere; eyes with abundant short hairs, about 0.5 width of ocellus in length; head lightly sericeous.

Thorax. (11.) Anterior surface of pronotum with few punctures, forming an obtuse angle with dorsal surface, distinctly separated from dorsal surface by keel; pronotal keel low, acute, extending almost to ventral extremity of pronotum, keel distinctly higher on humeral angle, projecting cephalad; humeral angle not well developed, rounded; dorsal surface evenly curved onto lateral surface, swollen posteriorly, with large, deep, contiguous punctures; lateral surface narrow, distinct from anterior surface, with few deep punctures; pronotal lobe wide, distinct. (12.) Scutum about .72 times as long as wide, with very large, deep, contiguous punctures. (13.) Scutellum rounded, posterior margin angular; in dorsal view 3 times as wide as long at middle, moderately bilobed, about 0.8 times as long at middle as at lateral margins, lateral margins rounded, posterior margin curved; in posterior view about 3.5 times as wide as height at middle, dorsal margin rounded laterally, slightly indented medially; in profile scutellum rounded, prominent, high, projecting over the plane of metanotum and up to or above plane of scutum; dorsal surface strongly convex, sloping dorsad posteriorly, posterior surface flat or very slightly concave; dorsal surface of scutellum rounded onto lateral surface; scutellar pocket slightly concave, pocket punctured posteriorly; dorsal surface with large, deep, contiguous punctures; posterior surface smooth, with few punctures dorsally; axillar ridge swollen, almost spheroid, broadly rounded, with small punctures. (14.) Metanotum 3.2 times as wide as long, slightly concave; dorsal margin bowed evenly dorsad, ventral margin bowed very slightly ventrad; surface smooth with few fine punctures. (15.) Mesopleuron strongly convex; anterior and posterior surfaces with scattered small punctures; punctures large medially, contiguous or nearly so. (16.) Dorsal sclerite of metapleuron 2.5 times as high as width at middle, surface rugose, with few large irregular punctures; secondary suture indistinct; first metapleural pit small, deep; ventral sclerite of metapleuron smooth, with few punctures; metapleural-propodeal suture scarcely evident. (17.) Propodeum angular; posterior surface with broad deep concavity, median area smooth, dorsolateral area with contiguous medium sized deep punctures; lateral surface with medium to large sized, deep punctures posteriorly, smaller and scattered anteriorly, surface rugose ventrally; lateral ridge low and irregular above propodeal angle, absent below angle, forming a round lobe on angle; propodeal angle well developed, in lateral view forming a rounded right angle, in posterior view forming large, rounded, lobe-like extension. (18.) Thorax with abundant, short, golden hairs on punctured surfaces, longest on scutellum; thorax lightly sericeous.

Abdomen. (19.) Tergum 1 flattened onto tergum 2, about 5.5 times as wide as long; sternum 1 about 6 times as wide as long at middle; tergum with few fine punctures, sternum rugose with sharp transverse, median ridge. (20.) Tergum 2 about .71 times as long as wide; high, evenly rounded in profile; with small deep punctures separated by about one diameter; punctures larger on sternum. (21.) Terga and sterna 3-5 punctured as on 2, more rugose anteriorly; tergum and sternum 6 without or with few small punctures. (22.) Abdomen with abundant, moderately long, golden hairs, directed caudad, lightly sericeous.

Coloration. As in *bilineolata* except wings often darker.

MALE. (1.) Wing length 6.5 mm.

As in female except for following:

Head. (2.) In frontal view .89 times as high as wide; in dorsal view .31 times as long as wide. (3.) Lateral ocellus separated from eye by .78 times distance between lateral ocelli and from occipital carina by .8 times this distance; vertex moderately convex, posterior surface gradually sloping ventrad. (4.) In lateral view gena .38 times as wide as eye at middle; postgenal convexity very slight, gena narrowed dorsally, about 1.6 times as wide at level of convexity as at level of eye emargination. (5.) Occipital carina low, rounded dorsally, high and acute laterally, extending to convexity, low and rounded on convexity. (7.) Clypeus .72 times as long as wide, slightly convex; distal margins curved; apical triangle about 1.2 times as long as width of antennal socket, apex broadly rounded; contact with eye equal to or little more than width of antennal socket; epistomal suture forming about a 30° angle with eye margin; clypeus entirely sericeous. (9.) Antenna with scape .53 times as long as width of clypeus; flagellum moderately swollen, eighth flagellomere 1.3 times as wide as long.

Thorax. (11.) Anterior surface of pronotum evenly curved onto dorsal surface, not distinctly separated from dorsal surface; pronotal keel low and rounded, extending little beyond humeral angle; humeral angle indistinct; dorsal surface evenly curved onto lateral surface; lateral surface not distinct from anterior surface. (13.) Scutellum more rounded; dorsal surface very convex, high, scutellum almost spheroid. (17.) Propodeal angles slightly swollen, more rounded in lateral view.

Abdomen. (24.) Spiculum very long, needlelike, at least 8 times as long as width at base.

Genitalia. (25.) Paramere 2.1 times as long as wide; apex slightly truncate, margins rounded; parameral spine about .5 times as wide at middle as at basal inflection, without distinct lateral invagination, without distinct notch at base of volsellar plate. (26.) Volsellar lobe long, very wide; in lateral view about 2 times as long as width at base; in ventral view extending to about middle of digital lobe, evenly tapered to a blunt apex; lobe strongly flattened. (27.) Cuspis appressed against paramere, apex rounded; cuspis with few black tubercles opposite base of digitus. In lateral view, digitus thick-set, rounded, about 2 times as long as width at base, posterior angle of digital lobe somewhat extended, rounded, not reaching apex of aedeagus, ventral angle round, directed anteriorly; in ventral view digital lobe ovoid, slightly wider basally than apically; digitus with small black tubercles laterally. (28.) Aedeagus in lateral view curved slightly ventrad, aedeagal lobe about 0.2 length of entire spatha, lobe moderately swollen; in ventral view, lobe evenly tapered to rounded apex, lobe about as wide as width of spatha at middle, inflected margins of lobe meeting at about middle of lobe, gradually divergent basally; spatha greatly expanded at base of lobe, to about twice width of spatha at middle, expansion with row of fine teeth along lateral margin; basal third of spatha with parallel margins, gradually narrowed at base; ventral hook long, curved laterad apically; aedeagal apodeme angular, widened apically, narrowed basally.

Coloration. Yellow markings more extensive than in female as follows: entire clypeus; ventral 0.5 of frons; scape except for small, dorsal, apical area; ventral surface of flagellum; ventral surfaces of coxae, trochanters and femora. Flagellum dark brown dorsally; mandible dull yellow basally.

Type Material. I have not been able to trace the type of this species. It is not in the Musée National in Paris and there are no specimens of *smithii* in Saussure's collection in Geneva. I am therefore designating a ♀ neotype for *Nectarinia smithii* Saussure. It bears the following label: "Carvoeiro, Rio

Negro-Rio Branco, Amazonas, 27-VIII-24". It agrees closely with the above description. The neotype is in the Museum of Comparative Zoology, Harvard.

VARIATION. In northern South America and Central America the occipital carina is very high on the postgenal convexity. In Central America the scutellum is low and does not project dorsad, and the punctures on the scutum are not as dense as in the Guianas. A similar situation is found in Mato Grosso and Acre where specimens may have both the high carina and low scutellum characteristic of *bilineolata* and *propodealis* and the deep, dense punctures characteristic of *smithii.* These wasps cannot be placed in any of the three species concerned and may be either an undescribed species or hybrids between *smithii* and *propodealis.*

The coloration in South America is relatively stable, the most common form having a wide discal band on the second tergum and two parallel lines on the scutum. The discal band varies somewhat but was only occasionally absent in the specimens examined.

In Costa Rica the color variation is similar to that of *bilineolata* and appears to be correlated with altitude. Specimens of the central plateau, altitude 1200 m, are dark with yellow maculations reduced, but wasps of the lowlands have the full complement of yellow markings as found in South America.

DISTRIBUTION. *Brachygastra smithii* extends from southern Mexico to southern Brazil. In South America it ranges over much of the Amazon, extending west to the Andes in Peru and Bolivia and south as far as the State of São Paulo. In its entire South American distribution it is sympatric with *bilineolata* and in western South America it occurs together with other species of the *smithii* group as well.

I have seen specimens from the following localities: CENTRAL AMERICA. *Costa Rica.* Prov. Cartago: 11 mi. SW. Cartago. Prov. Guanacaste: Playas del Coco. Prov. Puntarenas: Golfito; 34 km. S.E. Potrero Grande. Prov. San José: San José. *Guatemala.* Dept. Baja Verapáz: Salama. *Honduras.* Dept. Tegucigalpa: Tegucigalpa. *Mexico.* Est. Chiapas: 4 mi. S.W. Simojovel. *Panamá.* Dept. Colón: Portobelo.

SOUTH AMERICA. *Bolivia.* Dept. Beni: Cavinas on Río Beni. *Brazil.* Est. Acre: Iquiri. Est. Amazonas: Hyutanahan on Rio Purus; Rio Juruá; Tabatinga. Est. Mato Grosso: Utiariti on Rio Papagaio; Capitão Vasconcelos on Rio Tuatuari. Est. Pará: Obidos; Santarém. Terr. Roraima [= Terr. Rio Branco]: Viste Alegre. Est. São Paulo: (Eng. Cesar de Souza). *Colombia.* Dept. Boyaca: Muzo, 900 m. Dept. Magdalena: Río Frio. Dept. Santander: Puerto Olaya, 100 m. *French Guiana.* Dept. Guyane: Cayenne; Noveau Chantier; St. Jean du Maroni. *Perú.* Dept. Loreto: Río Marañon; Río Napo; Río Putamayo. *Surinam.* Dist. Nickerie: Sipaliwini.

BIOLOGY. A nest of "*bilineolata* var. *smithii*" has been figured by Ducke (1904, 1905), and other figures (Buysson, 1905; Ducke, 1910) are reproductions of his first figure. It is difficult to assign this nest to either *smithii* or *bilineolata*, but it is most likely the former species. Ducke (1910) reports that the nest is not large and has not been seen with more than four combs.

Bodkin (1917) reports a nest from British Guiana which was ¾ in. deep and 1½ in. greatest diameter. The lateral entrance hole is in the form of a vertical slit and opens into the lowermost combs.

Brachygastra propodealis Bequaert

Brachygastra bilineolata var. *propodealis* Bequaert, 1942. Jour. New York Ent. Soc. 50:305 (♀ holotype, Rio Marañon, Perú; in Museum Comparative Zoology, Harvard).

This species is distinct on the basis of the low scutellum, the high occipital carina and the moderately punctured scutum, but is almost identical to *bilineolata* and *smithii* in color and size. Its occurrence together with these species in northeastern Peru and southern Bolivia and the persistence of the above characters throughout its range indicates that it is a distinct species rather than a variety.

The scutellum of this species is very short and wide and does not project over the plane of the metanotum as in *smithii*. *B. propodealis* can be distinguished from *bilineolata* by the moderately punctured scutum and the rounded propodeal angles.

FEMALE. (1.) Wing length 6.37 mm.

Head. (2.) In frontal view .90 times as high as wide; in dorsal view .39 times as long as wide; posterior margin slightly curved. (3.) Lateral ocellus separated from eye by about 1.26 times distance between lateral ocelli and from occipital carina by 1.26 times this distance; vertex with small punctures separated by about one diameter; vertex moderately convex, posterior surface sloping slightly ventrad in profile. (4.) In lateral view gena about 1.3 times as wide as eye at middle; postgenal convexity very large, broad, posterior margin of convexity flattened medially; gena about twice as wide at level of convexity as at level of eye emargination; punctures small, separated by one diameter or less dorsally, more scattered ventrally, very small or absent on convexity. (5.) Occipital carina high, acute dorsally, slightly higher laterally, forming a very high blade-like flange on postgenal convexity, flange perpendicular to surface of convexity and equal to width of ocellus in height. (6.) Frons with medium sized punctures separated by about one diameter. (7.) Clypeus about 1.5 times as wide as long, moderately convex; distal margin straight, broadly rounded onto lateral lobe; apical triangle very long, about 1.5 times as long as width of antennal socket, narrowly rounded; contact with eye equal to about width of antennal socket; epistomal suture forming about a 45° angle with margin of eye, dorsally broadly curved; surface smooth, shiny, with few, scattered, small punctures, basal 0.2 lightly sericeous. (8.) Malar space 0.8 width of antennal socket. (9.) Antenna with flagellum moderately swollen, eighth flagellomere 1.6 times as wide as long. (10.) Head with abundant, very long, golden hairs, about as long as width of ocellus on vertex, shorter elsewhere; eyes with abundant short hairs, little less than width of ocellus in length; head very lightly sericeous.

Thorax. (11.) Anterior surface of pronotum with very few punctures dorsally, forming an obtuse angle with dorsal surface in profile, distinctly separated from dorsal surface by keel; pronotal keel low and rounded medially, higher and acute on humeral angle, extending to pronotal lobe, indistinct on lateral surface; humeral angle not well developed, rounded; dorsal surface evenly rounded onto lateral surface, slightly swollen posteriorly, with medium sized punctures separated by about one diameter; lateral surface very narrow, almost absent, not distinctly separated from anterior surface; pronotal lobe wide, distinct. (12.) Scutum about .75 times as long as wide with medium sized punctures widely spaced medially, separated by about one diameter laterally. (13.) Scutellum slightly rounded, margins more or less angular; in dorsal view scutellum about 3 times as wide as length at middle, lateral margins rounded, posterior margin curved; in posterior view 4 times as wide as height at middle, lateral margins rounded dorsally, dorsal margin strongly indented medially; in profile, scutellum slightly rounded, low, not projecting over plane of metanotum; dorsal surface flat, sloping dorsad posteriorly, posterior surface concave medially; scutellar pockets concave, dorsal surface not rounded onto lateral surface, pockets with few punctures posteriorly; dorsal surface with medium sized, shallow punctures contiguous or nearly so, posterior surface smooth ventrally with medium sized, shallow punctures dorsally; axillar ridge slightly swollen, narrowly rounded, with small punctures. (14.) Metanotum 2.6 times as wide as long, surface flat; dorsal margin slightly bowed dorsad, ventral margin slightly bowed ventrad; surface smooth with few fine punctures. (15.) Mesopleuron strongly convex; anterior and posterior surfaces with scattered small punctures; punctures large, contiguous dorsomedially, medium sized, separated by one diameter medially. (16.) Dorsal sclerite of metapleuron 3.3 times as high as width at middle; secondary suture evident as a small furrow; first metapleural pit small, shallow; ventral sclerite of metapleuron smooth; metapleural-propodeal suture evident as a shallow suture. (17.) Propodeum moderately angular; posterior surface broadly, slightly concave, median area with slightly irregular surface, dorsolateral area with small, deep, contiguous punctures; lateral surface with small to medium sized, deep punctures posteriorly, smaller more scattered ones anteriorly, surface rugose ventrally; lateral ridge irregular, low, extending little beyond propodeal angle, slightly higher on propodeal angle; propodeal angle moderately well developed, appearing as an obtuse angle in lateral view, rounded in posterior view. (18.) Thorax with abundant, long, golden hairs on punctured surfaces, longest on dorsum; thorax lightly sericeous.

Abdomen. (19.) Tergum 1 flattened onto tergum 2, in dorsal view 5.5 times as wide as long; sternum 1 about 5 times as wide as length at middle; tergum with few fine punctures; sternum rugose. (20.) Tergum 2 about .70

times as long as wide, high, evenly rounded in profile; tergum and sternum with evenly spaced, small, deep punctures separated by one to two diameters. (21.) Terga and sterna 3-5 punctured as on 2, more rugose anteriorly; tergum and sternum 6 with few punctures. (23.) Abdomen with abundant, short, golden hairs, lightly sericeous.

Coloration. Black with extensive yellow markings as follows : wide apical and lateral margins of clypeus; interantennal area; wide margin of inner orbit; vertex except for lateral margins of ocular swelling; anterior half of gena; ventral surface of scape; dorsal and lateral surface of pronotum; two medially narrowed, longitudinal bands on scutum; scutellum; metanotum; large rectangular, subtegular spot on mesopleuron; lateral portions of posterior surface of propodeum; ventral surfaces of coxae and tibiae; wide apical bands on terga 1-5 and sterna 2-5; wide discal band on tergum 2; anterior margin of sternum 2; apices of tergum and sternum 6. Mandible light brown, flagellum and legs dark brown.

VARIATION. The color pattern of *propodealis* is similar to that of *smithii* and *bilineolata* but differs in having the posterior surface of the propodeum almost entirely yellow. It is interesting to note that the extent of the scutal lines is not at all correlated with the development of the discal band of the second tergum, whereas in the other species of this group, loss of these lines is usually associated with loss of the discal band and an over-all reduction of yellow pigmentation.

DISTRIBUTION. *Brachygastra propodealis* is known only from the headwaters of the Amazon, extending south from northeastern Peru to northern Bolivia. Throughout much of its range it is found together with other species of the *smithii* group. In northeastern Peru, for example, it occurs together with *bilineolata, buyssoni* and *smithii.* Its range, therefore, is sympatric with all these species but does not appear to be as extensive as any of them.

Specimens have been examined from the following localities: *Bolivia.* Dept. Beni: Rurrenabaque on Río Beni. Dept. Chochabamba: Río Chapare, Tropical Region, 400 m. Dept. La Paz: Tumupasa. Brazil. Est. Guaporé: Pôrto Velho. Perú. Dept. Loreto: Middle Río Marañon. Dept. Huánuco: Monzon Valley, Tingo María; Pucallpa and Aguaytia; Tingo María. Dept. Pasco: Iscozazín.

Brachygastra buyssoni (Ducke) new combination
(Figs. 50, 66)

Nectarinia buyssoni Ducke, 1905. Rev. Ent. (Caen) 24:11.

Ducke's description, "scutello fortiter exciso et prominente, segmento mediano valde concavo et compressobidentato," leaves little doubt as to the identity of this interesting, seldom seen species. It is a large (7 mm.), black species with pale yellow markings similar to those of *smithii* and *bilineolata.* As Ducke noted, it is unique in the extreme development of the scutellum and propodeal angles.

Brachygastra buyssoni superficially resembles other species of the *smithii* group because of its similar color pattern, but it is larger and is marked with a light yellow, almost white pattern, while the other species have a distinct yellow. The propodeum is produced laterally into very large triangular projections (Fig. 66), the propodeal angles, and the scutellum is prominent, projecting over the plane of the metanotum and above the plane of the scutum. In addition the postgenal convexity is very large and bears the curved, blade-like occipital carina (Fig. 50). Although the latter characters are well developed in both *baccalaurea* and *smithii,* the convexity and carina are never as large as in *buyssoni.* Unusually dark specimens from higher elevations may resemble *lecheguana* but examination of the above mentioned characters facilitates separation of these species.

FEMALE. (1.) Wing length 6.85 mm.

Head. (2.) In frontal view .87 times as high as wide; in dorsal view about .42 times as long as wide; posterior margin moderately curved. (3.) Lateral ocellus separated from eye by about 1.4 times distance between lateral ocelli and from occipital carina by about 1.6 this distance; vertex with medium sized punctures separated by one diameter or less posterior to eye, widely spaced lateral to lateral ocelli; vertex slightly convex, posterior surface sloping slightly ventrad. (4.) In lateral view gena about as wide as eye in middle; postgenal convexity very large, round; gena about 1.75 times as wide at level of convexity as at level of eye emargination; punctures medium sized dorsally, separated by two diameters or less, diminishing in size ventrally, very fine and widely spaced at level of convexity. (5.) Occipital carina low, rounded, more or less distinct dorsally, extending onto postgenal convexity laterally, forming a wide, curved, blade-like flange, as high as or higher than width of ocellus, perpendicular to surface of gena, terminating at mandibular condyle. (6.) Frons with medium sized punctures separated by one diameter. (7.) Clypeus about 1.7 times as wide as long, moderately convex; distal margin straight, narrowly rounded onto lateral lobe; apical triangle large, about 1.5 times width of antennal socket in length, apex narrowly rounded; contact with eye equal to about 0.5 width of antennal socket; epistomal suture forming about a 60° angle with eye margin, dorsally indistinct, forming a flattened V; clypeal surface smooth, shiny, with few, scattered fine punctures, basal 0.2 sericeous. (8.) Malar space about 0.7 times width of antennal socket. (9.) Antenna with flagellum moderately swollen, eighth flagellar segment about 1.5 times as wide as long. (10.) Head with short, erect, fine, white hairs as long as width of ocellus on vertex, much shorter elsewhere; eye with very short hairs; head lightly sericeous.

Thorax. (11.) Anterior surface of pronotum smooth, shiny, with few small scattered punctures, in profile forming a right angle with dorsal surface, distinctly separated from dorsal surface; pronotal keel low medially, rounded below humeral angle, developed into high ridge inflected cephalad at humeral angle; humeral angle rounded in dorsal and lateral views; dorsal surface evenly curved onto lateral surface, with large, deep punctures, almost contiguous anteriorly, separated by one diameter near posterior margin; lateral surface narrow, with few medium sized punctures; pronotal lobe wide, indistinct. (12.) Scutum about .75 times as long as wide; punctures large, deep, contiguous or nearly so anteriorly, separated by two diameters or less posteriorly. (13.) In dorsal view scutellum about 3 times as long as width at middle, strongly bilobed, about .75 times as long at middle as at lateral margin, posterior margin forming a flattened V; in posterior view about 4 times as wide as height at middle, dorsal margin bowed strongly dorsad laterally; in profile scutellum projecting posterodorsally as high as scutum, dorsal surface forming a distinct acute angle with posterior surface; dorsal surface slightly convex with large deep contiguous punctures, posterior surface flat, smooth, with scattered medium sized punctures; scutellar pocket small, heavily punctured apically; axillar ridge short, swollen, almost spheroid, punctured. (14.) Metanotum about 3 times as wide as long, slightly concave medially; dorsal margin evenly bowed dorsad, ventral margin very slightly bowed ventrad; surface smooth with few small punctures. (15.) Mesopleuron strongly convex; with medium sized punctures separated by one diameter or less dorsally, smaller ventrally; anterior and posterior surfaces with few small punctures. (16.) Dorsal sclerite of metapleuron 3 times as high as wide at middle, with few medium sized punctures; secondary suture indistinct; first metapleural pit small, shallow; ventral sclerite with few small punctures, metapleural-propodeal suture evident as shallow furrow. (17.) Propodeum greatly developed, angular; entire posterior surface strongly concave, finely rugulose; dorsolateral areas with large, contiguous, shallow punctures; lateral surface with large, contiguous punctures posteriorly, smaller ones anteriorly, few ventrally; lateral ridge irregular on propodeal angle, distinct below propodeal angle; propodeal angle greatly developed forming a posterolateral triangular projection about as long as height of propodeum. (18.) Thorax with short, erect golden hairs in punctured areas, longer on scutum and propodeum than elsewhere; thorax lightly sericeous.

Abdomen. (19.) Tergum 1 flattened onto tergum 2, scale-like, almost 5 times as wide as long in dorsal view; sternum 1 about 5 times as wide as long in dorsal view; tergum with small scattered punctures, sternum rugose with acute, transverse ridge. (20.) Tergum 2 about .72 times as long as wide, high, abruptly convex in profile; with dense, evenly spaced, small, deep punctures separated by about one diameter; sternum 2 with small punctures more widely spaced. (21.) Terga and sterna 3-5 with small punctures separated by about three diameters; tergum and sternum 6 with few fine punctures.

(22.) Abdomen with short, golden hairs directed caudad, lightly sericeous.

Coloration. Black with light yellow markings as follows: two interantennal spots mediodorsal to antennal sockets; lateral and distal margins of clypeus; inner orbit from epistomal suture to emargination; wide band extending from mandibular articulation to median genal area; two elongate spots posterolateral to posterior ocelli forming a flattened V interrupted medially; median edge of pronotal keel; small spot on humeral angle; posterior tips of pronotum; subtegular spot on mesopleuron; axilla; dorsal half of metanotum; narrow apical margins of abdominal terga 1-6; narrow apical margins of sterna 2-5; narrow transverse discal band on tergum 2. Wings clear, veins dark brown.

TYPE MATERIAL. The holotype, a female from Tabatinga, Amazonas, Brazil, collected by Ducke, may be in the Museu Goeldi, Belém, Brazil. Additional specimens collected by Ducke are in the Musée National d'Histoire Naturelle, Paris.

DISTRIBUTION. The species was originally believed to be restricted to the headwaters of the Amazon but I have seen a specimen from the Rio Xingu in Mato Grosso which unmistakably belongs to this species. Additional specimens from Bolivia and Peru indicate its wide distribution.

Specimens have been examined from the following localities: *Brasil.* Est. Mato Grosso: Aldeia Juruna on the Rio Xingu. *Perú.* Dept. Huánuco: Tingo María. Dept. Loreto: Middle Río Ucayali.

In the literature, references are found to the following localities: *Bolivia.* Dept. La Paz: Tumupasa (Bequaert, 1932). *Brasil.* Est. Amazonas: Tabatinga (Ducke, 1905). *Perú.* Dept. Loreto: Iquitos (Ducke, 1908).

The *B. lecheguana* Group

The species of this group have the following common characteristics: occipital carina not well developed on ventral half of gena; postgenal convexity absent or very weak; pronotal keel low, not projecting cephalad on humeral angle; scutellum angular; color pattern relatively stable, yellow maculations only on frons and apical margins of abdominal segments. The nests of this group are large and often persist for several years.

The species of this group have been grouped under one species, *lecheguana*, in the literature. On the basis of male characters, the material is here divided as follows:

B. mellifica (Say) in Mexico and Central America north of Costa Rica; *B. lecheguana* (Latreille), widespread in South America; *B. borellii* (Zavattari) in the southern Andean regions.

Brachygastra mellifica (Say) new combination

(Figs. 22-24)

Polistes mellifica Say, 1837. Boston Jour. Nat. Hist. 1(4):390 (♀ ♂, near Jalapa, Mexico—lost; ♂ neotype, Veracruz, Mexico, by present designation in the collection of the University of California, Berkeley).

Nectarinia mellifica; Saussure, 1853-1858. Et. Fam. Vesp. 2:226, 232, 233.

Nectarina lecheguana; Buysson, 1905. Ann. Soc. Ent. France 74:542, 547, 558, pl. 11, figs. 1-7, pl. 12, figs. 1-3, 6-8, 10, 13, pl. 15, 16 (in part).
Nectarina mellifica; Smith, 1857. Cat. Hymen. Brit. Mus. 5:137.
Nectarina mellifera; Dalla Torre, 1904. *in* Wytsman, Gen. Insect., fasc. 19:86.
Caba lecheguana; R. von Ihering, 1904. Rev. Mus. Paulista 4:106, 109 (in part) (misidentification).
Caba (Nectarina) mellifica; Barber, 1905. Proc. Ent. Soc. Washington 7:25.
Nectarina lecheguana var. *velutina;* Buysson, 1905. Ann. Soc. Ent. France 74:547, 563 (in part).
Chartergus aztecus Cameron, 1906. Invertebrata Pacifica 1:154 (♀, Mexico; in British Museum).
Chartergus arizonaensis Cameron, 1907. Invertebrata Pacifica 1:181, 182 (♀, Nogales, Arizona; in British Museum).
Chartergus centralis Cameron, 1907. Invertebrata Pacifica 1:181, 182 (2 ♀♀, Chimandega, Nicaragua and Champerico, Guatemala; in British Museum).
Nectarina cameroni Meade-Waldo, 1911. Ann. Mag. Nat. Hist. (8)7:112. New name for *Chartergus aztecus* Cameron, 1906.
Brachygastra lecheguana; Bequaert, 1944. Bull. Mus. Comp. Zool. 94:271, 272 (in part).
Brachygastra lecheguana var. *velutina;* Richards and Richards, 1951. Trans. R. Ent. Soc. London 102:26 (in part).

Brachygastra mellifica has long been considered a synonym of *lecheguana* and all references to the latter species north of Panama are references to *mellifica*. Although the females are often very similar and difficult to separate, the male genitalia of *mellifica* are distinct from those of *lecheguana* on the basis of the elongate digital lobe of the volsella (Fig. 23). No intermediates have been found, and the few male specimens of *lecheguana* seen from Panama are distinctly different from males of *mellifica* from Honduras.

The only positive way of separating *mellifica* from *lecheguana* is on the basis of male characters. If males are unavailable, locality must serve as the diagnostic character. The majority of females of *mellifica* differ from *lecheguana* by the almost straight posterior margin of the scutellum, but this character varies in both species, particularly in the latter.

FEMALE. (1.) Wing length 7.44 ± .242 mm.

Head. (2.) In frontal view .87 times as high as wide; in dorsal view .43 times as long as wide; posterior margin slightly curved, almost straight. (3.) Lateral ocellus separated from eye by 1.25 times distance between lateral ocelli and from occipital carina by 1.25 times this distance; vertex with small punctures separated by about one diameter, occasionally contiguous posterior to eye; vertex strongly convex, posterior surface sloping ventrad in profile. (4.) In lateral view gena about as wide as eye at middle; postgenal convexity absent, posterior margin curving gradually towards mandibular condyle; gena about as wide on ventral half as at level of eye emargination; punctures small, scattered, occasionally contiguous or forming long rows, slightly smaller ventrally. (5.) Occipital carina low, acute, becoming lower, occasionally indistinct on ventral third of gena. (6.) Frons with small, deep punctures separated by about one diameter or less. (7.) Clypeus about 1.6 times as wide as long, slightly convex; distal margin curved, narrowly rounded onto lateral lobe; apical triangle about as long as width of antennal socket, apex broadly rounded; contact with eye equal to about 0.8 times width of antennal socket; epistomal suture forming about a 60° angle with margin of eye, curved slightly ventrad medially; surface smooth, shiny, with widely spaced, small punctures, basal 0.3 lightly sericeous, distal marginal hairs sparse, longer apically than laterally. (8.) Malar space about 0.3 times width of antennal socket. (9.) Antenna with flagellum moderately swollen, eighth flagellomere 1.5 times as wide as long. (10.) Head with abundant, long, erect, golden hairs, recurved distally, little longer than width of ocellus on vertex, shorter elsewhere; eye with abundant, short, erect, golden hairs; head golden sericeous.

Thorax. (11.) Anterior surface of pronotum with scattered small punctures dorsally, distinctly separated from dorsal surface; pronotal keel, low, obtuse medially, higher and acute laterally, extending to humeral angle, absent on lateral surface; humeral angle developed into a distinct angular shoulder projecting cephalad; dorsal surface abruptly rounded onto lateral surface, with deep, medium sized punctures separated by about one diameter; lateral surface narrow, not distinctly separated from anterior surface, with small to medium sized punctures separated by about one diameter; pronotal lobe wide, distinct. (12.) Scutum .85 times as long as wide with evenly spaced, small punctures separated by about one to two diameters. (13.) In dorsal view scutellum about 2 times as wide as long, very slightly bilobed, only slightly longer at lateral margin than at middle, posterior margin almost straight; in posterior view about 5 times as wide as height at middle, dorsal margin straight; in profile scutellum angular, projecting slightly over plane of metanotum, not projecting above plane of scutum; scutellar pocket flat, punctured; dorsal surface slightly convex; posterior surface slight concave; dorsal surface with medium sized shallow punctures separated by about one diameter anteriorly, contiguous posteriorly; posterior surface smooth with few punctures dorsally; axillar ridge wide, swollen, with small punctures. (14.) Metanotum about 3 times as wide as long, flat; dorsal margin bowed evenly dorsad, occasionally forming slightly rounded lip, ventral margin slightly curved; surface with small punctures dorsolaterally. (15.) Mesopleuron moderately convex with scattered small punctures on anterior and posterior surfaces; punctures large to medium sized medially, surface rugose, irregularly punctured dorsally. (16.) Dorsal sclerite of metapleuron about twice as wide as high with scattered small punctures; secondary suture indistinct; first metapleural pit wide, deep; ventral sclerite of metapleuron with few fine punctures; metapleural-propodeal suture evident as a shallow furrow. (17.) Propodeum angular; posterior surface with narrow, moderately deep concavity without punctures, dorsolateral area irregularly punctured, rugose; lateral surface with small to medium sized punctures separated by about one diameter; lateral ridge low, irregular below propodeal angle; propodeal angle slightly swollen, bearing a prominent blade-like, rounded lobe, acutely projecting in lateral view, forming round lateral lobe in posterior view. (18.) Thorax with abundant long golden hairs, recurved distally; densely golden sericeous.

Abdomen. (19.) Tergum 1 distinctly set off from tergum 2, about 6 times as wide as long in dorsal view; sternum 1 about 5 times as wide as long; tergum with fine punctures, sternum rugulose. (20.) Tergum 2 about .80 times as long as wide; low, abruptly convex in profile with very small punctures, separated by about two to four diameters, larger laterally than medially; sternum 2 with punctures as on tergum. (21.) Terga and sterna 3-5 with widely spaced, small punctures, tergum and sternum 6 with few punctures. (22.) Abdomen with abundant, short, curved hairs, directed caudad, lightly sericeous.

Coloration. Black with yellow markings more or less developed as follows; small area on lower inner orbit; median area of pronotal keel; portions of dorsal margin of metanotum; narrow apical bands on terga 1-5 and sterna 2-5; tergum and sternum 6. Mandible, antenna and legs dark brown. Wings dark yellow, blackened apically.

MALE. (1.) Wing length 7.52 ± .212 mm.

As in female except for following:

Head. (2.) In frontal view .86 times as high as wide; in dorsal view .37 times as long as wide. (3.) Lateral ocellus separated from eye by about .78 times distance between lateral ocelli and from occipital carina by 0.9 times this distance; vertex strongly convex, posterior margin almost vertical. (4.) Gena .43 times as wide as eye in lateral view; postgenal convexity very slight; gena about as wide at level of convexity as at level of eye emargination. (5.) Occipital carina low, rounded, extending about 0.6 length of gena, absent ventrally. (7.) Clypeus 1.3 times as wide as long, slightly convex; distal margin curved; apical triangle slight, about 0.5 times as long as width of antennal socket, apex broadly rounded; contact with eye equal to about width of antennal socket; epistomal suture forming about a 30° angle with eye margin; clypeus entirely sericeous. (8.) Malar space .2 times width of antennal socket. (9.) Antenna with scape .55 times as long as width of clypeus; flagellum moderately swollen, eighth flagellomere 1.5 times as wide as long.

Thorax. (11.) Pronotal keel low, ending abruptly at humeral angle; humeral angle not developed into distinct shoulder, forming a slight conical swelling, dorsal surface curved evenly onto lateral surface. (13.) Scutellum with dorsal surface more convex; punctures larger, deeper; scutellar margins more rounded. (17.) Propodeum more rounded; propodeal angles obtuse, not acutely projecting.

Abdomen. (24.) Spiculum long, pointed about 2.3 times as long as width at base.

Genitalia. (25.) Paramere 2.2 times as long as high, apex roundly truncate; parameral spine about 0.5 times as wide at middle as at basal inflection, with lateral invagination; shallow notch at base of volsellar plate. (26.) Volsellar lobe very long, attenuate, 5 times as long as width at base, slightly tapered, apex rounded; lobe curved laterally, slightly depressed, extending little beyond middle of digital lobe. (27.) Cuspis flattened against paramere, pointed in lateral view; cuspis bearing few black tubercles opposite base of digitus. In lateral view digitus elongate, pointed, about 3 times as long as wide at base, posterior angle of digital lobe greatly extended, almost reaching apex of aedeagus, ventral angle produced into a long pointed ventral projection; in ventral view posterior angle long, pointed; ventral angle rounded, projecting laterally; digitus with small black tubercles laterally. (28.) Aedeagus in lateral view curved evenly ventrad, aedeagal lobe about 0.3 length of entire spatha, lobe slightly swollen; in ventral view lobe about 1.8 times as wide at apex as at base, lobe at most 2 times as wide as width of spatha at middle, lobe with lateral margins inflected mesad about 0.4 width of lobe; inflected margins parallel along middle of lobe, slightly divergent elsewhere; ventral hook short, wide, triangular, curved slightly laterad apically; aedeagal apodeme with ventral swelling forming a right angled ventral margin, dorsal margin evenly curved.

Coloration. Black with yellow markings more or less developed as follows: inner orbits; two interantennal spots; area ventral to antennal sockets; distal margin of clypeus; ventral surface of scape; base of mandible; pronotal keel; tegula; axillar ridge; portions of dorsal surface of scutellum; anteroventral surfaces of coxae; portions of ventral surfaces of trochanters and tibiae; apical bands on terga 1-6; sterna 2-6; tergum and sternum 7. Flagellum light ferruginous. Apex of mandible and legs dark brown.

Type Material. As virtually all of Say's types have been lost, apparently including that of *mellifica*, I am designating a neotype for *Polistes mellifica* because of the similarity of this species to *lecheguana*. The neotype is a male with the following labels: Vera Cruz, V.C., Mex., VI-20-51; P. D. Hurd collector. The genitalia have been dissected and are in a small vial attached to the pin. It is in the collections of the University of California at Berkeley.

Variation. Characters that vary in *B. mellifica* include the height of the occipital carina, the posterior margin of the scutellum, and the lateral ridge of the propodeum. The variation of these characters is slight and occurs throughout the range and at any one locality. The posterior scutellar margin is straight in the majority of individuals but may be slightly V-shaped or broadly concave. In western Costa Rica the margin may be distinctly V-shaped but is rarely as emarginate as it is in *lecheguana* in central Panama where it reaches the extreme condition for the *lecheguana* group. The development of the scutellum appears to be correlated with the lobe of the lateral ridge of the propodeum.

The majority of specimens of *mellifica* have narrower abdominal bands than *lecheguana* but the width of the bands is variable. The bands are distinctly narrower in western and central Mexico than on the eastern coast of Mexico and in the countries to the south. In Mexico the size of the facial maculations is correlated with the width of the bands but in Central America these maculations may be lacking or reduced in wasps which have well developed maculations on the scutellum, metanotum and abdomen.

DISTRIBUTION. *Brachygastra mellifica* extends from southern Texas and Arizona to western Costa Rica and possibly western Panama. It is not found in the dry regions of northern Central Mexico, but is very common in the coast states and the southern half of Mexico.

Specimens have been examined from the following localities: CENTRAL AMERICA. *British Honduras.* Belize; Corozal. *Costa Rica.* Prov. Alajuela: San Fernando. Prov. Guanacaste: Filadelfia; 18 km E. Liberia; Playas del Coco. Prov. Puntarenas: Barranca; Puntarenas. *El Salvador.* Dept. San Salvador: 4 mi. S., 5 mi. N., and Quezaltepeque; San Salvador. *Guatemala.* Dept. Chilmaltenango: Pochuta, 1000 m. Dept. Escuintla: San José. Dept. Guatemala: Guatemala City. Dept. Jutiapa: Tiucal. Dept. Santa Rosa: Amatitlán. Dept. Suchiteqequez: Moca, 1000 m. Dept. Vera Paz: Salamanca. (Nueva Concepción; Yepocapa). *Honduras.* Dept. Tegucigalpa; Zamorano, 20 mi. from Tegucigalpa. Dept. Yoro: Subirana.

NORTH AMERICA. *Mexico.* Many localities in the following states: Campeche, Chiapas, Colima, Guerrero, Hidalgo, Jalisco, Mexico, Michoacán, Morelos, Nuevo León, Oaxaca, Puebla, San Luís Potosí, Sinaloa, Sonora, Tamaulipas, Veracruz, Yucatán. *United States.* Arizona. Nogales. Texas. Cameron Co.: Brownsville, Rio Hondo. Hidalgo Co.: Edinburg, McAllen, Mission, Progresso, Weslaco. Kleberg Co.: Kingsville.

BIOLOGY. The biology of this species has been summarized by Bequaert (1932) with *Nectarina lecheguana.* This wasp, like *lecheguana*, has long been known and often been cultured for its honey. Saussure gave the first descriptions and figures of the nest of this species. The nest is spherical and often built incorporating one or several supporting branches. The envelope is of tough paper and has irregular patches of shallow cells which are destined to become the outermost comb covered by yet another envelope. The interior structure is a modified type of the phragmocyttarous arrangement, spherical phragmocyttarous of Saussure. The arrangement is such that the combs are strongly convex, almost spherical with the cells opening outward. Although it appears to be a series of spherical combs, one inside the other, it is, in fact, a single comb extended in a spiral fashion, *i.e.*, the combs are continuous. Passageways between the various levels continue this spiral pattern as short continuous ramps. This arrangement provides a very efficient utilization of space, and enables a very large population to occupy a relatively small space. Schwarz (1929) reported a nest with a population of about 15,000 and I have seen a colony of an estimated 10,000 individuals.

The colonies are perennial and may persist many years. In Mexico the nests are kept for the honey which is taken annually by removing all but the uppermost portions of the nest. The wasps then rebuild the nest on the old base. The activity of a colony throughout the year is quite seasonal. Schwarz reported that in January, in Texas, nests had no larvae or honey, and I have seen a colony in August in Costa Rica in a similar condition. Perhaps the activity of reproduction is restricted to the most favorable season during which both larval food and nectar are available. The wasps are not dormant during the unfavorable season but remain active in the empty nest and in the field.

Brachygastra lecheguana (Latreille)
(Figs. 1-9, 13, 15-18)

Vespa sericea Fabricius, 1804. Syst. Piezat., p. 266 (♀, South America; lectotype in Universitettes Zoologiske Museum, Copenhagen, by present designation). Not *Vespa sericea* Olivier, 1791.

Polistes lecheguana Latreille, 1824. Mem. Mus. Hist. Nat., Paris 11:317 (♀, Brazil).

Brachygastra analis Perty, 1833. Delectus Anim. Artic. Brasil., p. 146 (no sex given, State of Piauí, Brazil).

Nectarina analis; Swainson and Shuckard, 1840. On the History and Natural Arrangement of Insects, p. 183.

Vespa lecheguana; Swainson and Shuckard, 1840. On the History and Natural Arrangement of Insects, p. 183.

Brachygastra velutina Spinola, 1841. Ann. Soc. Ent. France 10:126, pl. 3, fig. 5 (2 ♀♀, Cayenne, French Guiana; in Museo di Zoologia, Torino). New synonymy.

Melissaia lecheguana White, 1841. Ann. Mag. Nat. Hist. 7:320. New name for *Nectarinia lecheguana* (Latreille).

Epipone lecheguana; Curtis, 1844. Proc. Linn. Soc. London 1(20):188.

Brachygastra aurulenta Erichson, 1848. *in* Schomburgk, Reisen in British Guiana 3:590. New synonymy.

Nectarinia analis; Saussure, 1853-1858. Et. Fam. Vesp. 2:226, 230, 232.

Nectarinia binotata Saussure, 1853-1858. Et. Fam. Vesp. 2:230 (♂, Cayenne, French Guiana; in Musée National d'Histoire Naturelle, Paris; labeled Colombia).

Nectarinia lecheguana; Saussure, 1853-1858. Et. Fam. Vesp. 2:226, 231, 232, pl. 34, figs. 1, 3.

Nectarinia velutina; Saussure, 1853-1858. Et. Fam. Vesp. 2:226, 237.

Chartergus sericeus; Mobius, 1856. Abh. Naturw. Ver. Hamburg 3:144, pl. 15.

Nectarinia aurulenta; Smith, 1857. Cat. Hymen. Brit. Mus. 5:137.

Nectarina binotata; Smith, 1857. Cat. Hymen. Brit. Mus. 5:136.

Nectarina lecheguana; Smith, 1857. Cat. Hymen. Brit. Mus. 5:136.

Nectarina velutina; Smith, 1857. Cat. Hymen. Brit. Mus. 5:137.

Nectarinia sericea; Saussure, 1867. Reise der Novara, pp. 22, 23.

Caba analis; R. von Ihering, 1904. Rev. Mus. Paulista 4:107, 113.

Caba binotata; R. von Ihering, 1904. Rev. Mus. Paulista 4:107.

Caba lecheguana; R. von Ihering, 1904. Rev. Mus. Paulista 4:106, 109 (in part).

Nectarina lecheguana var. *binotata;* Buysson, 1905. Ann. Soc. Ent. France 74:547, 563.

Nectarina lecheguana var. *velutina;* Buysson, 1905. Ann. Soc. Ent. France 74:547, 563 (in part).

Caba lecheguana var. *velutina;* Zavattari, 1906. Boll. Mus. Anat. Comp. (Torino) 21(529):3, 4.

Brachygastra lecheguana; Bequaert, 1944. Bull. Mus. Comp. Zool. 94:271, 272 (in part).

Brachygastra lecheguana var. *velutina;* Bequaert, 1944. Bull. Mus. Comp. Zool. 94:272.

Because of the abundance and wide distribution of this species, there have been many references to it and its interesting nesting habits. Similarly, there have been numerous forms described and the synonymy is particularly lengthy. Bequaert (1932) gives an excellent history of this species but his synonymy does not separate *mellifica.*

B. lecheguana, one of the largest species of the genus (total length about 8 mm.), is a predominantly black wasp with yellow abdominal bands. It is similar to *mellifica* of North and Central America but has the scutellum usually more emarginate posteriorly (Figs. 9, 12). The males are distinct on the basis of the rounded, blunt digital lobe (Fig. 18) which in *mellifica* is attenuated posteriorly (Fig. 24).

Female. (1.) Wing length 7.33 ± .349 mm.

Head. (2.) In frontal view .85 times as high as wide; in dorsal view .43 times as long as wide; posterior margin slightly curved. (3.) Lateral ocellus separated from eye by 1.25 times distance between lateral ocelli and from occipital carina by 1.36 times this distance; vertex with small, contiguous punctures anterior to lateral ocelli, punctures medium sized, separated by about one diameter posterior to lateral ocelli; vertex strongly convex, posterior surface sloping ventrad in profile. (4.) In lateral view gena about 1.4 times as wide as eye at middle; postgenal convexity absent, ventral half of posterior margin of gena gradually curved, gena widest at middle; gena about as wide on ventral half as at level of eye emargination; punctures medium sized, separated by one to four diameters dorsally, smaller, more scattered ventrally. (5.) Occipital carina low, acute, of even height, extending to mandibular condyle. (6.) Frons with medium sized punctures separated by one diameter or less, often contiguous medially. (7.) Clypeus about 1.7 times as wide as long, slightly convex; distal margin curved, broadly rounded onto lateral lobe; apical triangle long, about as long as width of antennal socket; contact with eye equal to about 0.8 width of antennal socket; epistomal suture forming about a 60° angle with eye margin, dorsally forming a broadly flattened V; surface smooth, with scattered small punctures, basal 0.8 sericeous. (8.) Malar space about 0.4 width of antennal socket. (9.) Antenna with flagellum moderately swollen, eighth flagellomere about 1.5 times as wide as long. (10.) Head with abundant, erect golden hairs about as long as width of ocellus, slightly longer on vertex; eye with abundant short hairs about 0.5 times as long as width of ocellus; head densely golden sericeous.

Thorax. (11.) Anterior surface of pronotum with few scattered punctures dorsolaterally, distinctly separated from dorsal surface of pronotum; pronotal keel low, acute, slightly higher laterally than medially, extending to humeral angle only, absent on lateral surface; humeral angle developed into a distinct shoulder, rounded; dorsal surface abruptly rounded onto lateral surface, with deep, medium to large sized punctures separated by less than one diameter; lateral surface not distinctly separated from anterior surface, with large contiguous punctures dorsally, small ventrally; pronotal lobe wide, distinct. (12.) Scutum about 0.8 times as long as wide with evenly spaced, medium sized punctures separated by two to three diameters. (13.) Scutellum in dorsal view about 2.3 times as wide as long, moderately bilobed, about 0.8 as long at middle as at lateral margin, posterior margin forming a flattened V; in posterior view about 5.5 times as wide as height at middle, dorsal margin depressed medially, rounded laterally; in profile, scutellum angular, forming an acute angle projecting over plane of metanotum, not extending above plane of scutum; dorsal surface slightly convex, posterior surface slightly concave; scutellar pocket flat, punctured; dorsal surface with large, deep contiguous punctures, posterior surface with few medium sized punctures along dorsal margin; axillar ridge swollen, wide, with small punctures. (14.) Metanotum 3 times as wide as long, flat; dorsal margin bowed evenly dorsad, with slight rounded lip, ventral margin broadly sinuate; surface smooth with few small dorsolateral punctures. (15.) Mesopleuron moderately convex; anterior and posterior surfaces with scattered small punctures; large, shallow, contiguous punctures mediodorsally, punctures separated by about one diameter ventrally. (16.) Dorsal sclerite of metapleuron 2.5 times as high as width at middle, with small punctures separated by about one diameter; secondary suture indistinct; first metapleural pit wide, deep; ventral sclerite of metapleuron with scattered fine punctures; metapleural-propodeal suture evident as wide, shallow furrow. (17.) Propodeum angular; posterior surface with narrow, deep, median, smooth concavity, dorsolateral areas rugose; lateral surface with medium sized punctures, contiguous posteriorly, smaller and more scattered elsewhere, rugose ventrally; lateral ridge incomplete, present only on propodeal angles and immediately below; propodeal angle with compressed, blade-like rounded projection, forming a rounded right angled lobe in lateral view, an abruptly truncate, lateral shelf-like ridge in posterior view. (18.) Thorax with dense, short, golden hairs on punctured surfaces, densely golden sericeous.

Abdomen. (19.) Tergum 1, flattened, scale-like, not distinctly set off from tergum 2, about 6 times as wide as long in dorsal view; sternum 1 about 6 times as wide as long; tergum with few fine punctures laterally; sternum rugulose. (20.) Tergum 2 about .78 times as long as wide, distinctly wider than thorax in dorsal view; low, abruptly convex in profile, with evenly spaced very small punctures medially, becoming larger laterally; sternum 2 with small punctures separated by two to three diameters medially, closer laterally. (21.) Terga and sterna 3-5 with punctures as on 2; tergum and sternum 6 with few small punctures. (22.) Abdomen with abundant golden hairs of medium length, directed caudad; abdomen densely sericeous.

Coloration. Black with yellow markings more or less developed as follows: small, ventral spot on inner orbit; median area of pronotal keel; axillar ridge; dorsal margin of metanotum; wide apical bands on terga 2-5, sterna 2-5; tergum and sternum 6. Flagellum, apex of mandible, legs dark brown. Wings yellow to dark brown, apices darker.

MALE. (1.) Wing length 7.60 ± .417 mm.

As in female except for following:

Head. (2.) In frontal view .86 times as high as wide; in dorsal view .36 times as long as wide. (3.) Lateral ocellus separated from eye by .81 times distance between lateral ocelli and from occipital carina by .95 times this distance; vertex strongly convex, posterior surface almost vertical. (4.) Gena about .34 times as wide as eye in lateral view; postgenal convexity absent, gena slightly wider ventrally than dorsally. (5.) Occipital carina low dorsally, somewhat higher on dorsal 0.7 of gena, absent on ventral 0.3. (7.) Clypeus about 1.4 times as wide as long, moderately convex; distal margin curved, apex broadly rounded; apical triangle about 0.8 times width of antennal socket; contact with eye equal to about 1.5 times width of antennal socket; epistomal suture forming about a 30° angle with margin of eye; clypeus entirely sericeous. (8.) Malar space very short. (9.) Scape about 0.5 times width of clypeus; flagellum slightly swollen, eighth flagellomere about 1.25 times as wide as long.

Thorax. (11.) Pronotal keel high, acute, ending abruptly at humeral angle; humeral angle not developed into a distinct shoulder, at most forming a low, broadly conical convexity; dorsal surface sloping gradually onto lateral surface. (13.) Scutellum with dorsal surface convex, heavily punctured; scutellum more rounded. (17.) Propodeum more rounded; lateral ridge often well developed; propodeal angle less well developed, obtuse in lateral view.

Abdomen. (24.) Spiculum long, wide, evenly tapered to rounded apex, about twice as long as width at base.

Genitalia. (25.) Paramere about 2 times as long as high; apex truncate, rounded; parameral spine about 0.5 times as wide at middle as at basal inflection, with lateral invagination; paramere with wide, shallow notch at base of volsellar plate. (26.) Volsellar lobe long, finger-like, about twice as long as width at base, slightly tapered distally, apex rounded; lobe curved slightly laterad, depressed, extending to middle of digital lobe. (27.) Cuspis flattened against paramere, apex pointed; cuspis bearing black tubercules opposite base of digitus. In lateral view digitus thick-set, about twice as long as width at base, posterior angle of digital lobe not extended, forming a rounded right angle, ventral angle greatly produced forming a long ventral projection rounded apically; in ventral view posterior angle blunt, rounded, ventral angle rounded, projecting slightly laterad; digitus with many small black tubercules laterally. (28.) Aedeagus in lateral view evenly curved ventrad; aedeagal lobe about 0.3 length of entire spatha, lobe slightly swollen; in ventral view lobe little wider at apex than at base, lobe about twice as wide as width of spatha at middle, lobe with lateral margins inflected about .45 width of spatha almost touching medially, margins parallel at middle slightly divergent elsewhere; ventral hook long, narrow, curved laterad apically;

aedeagal apodeme wide basally, with rounded swelling ventrally, dorsal margin evenly curved.

Coloration. Black with yellow markings more or less developed as follows: inner orbit; subantennal area; median interantennal spot; distal margin of clypeus; ventral surface of scape; median area of pronotal keel; tegulae; portions of dorsal margin of metanotum; anteroventral surface of coxae and trochanters; portions of ventral surfaces of femora; apical margins of terga 1-6, sterna 2-6; tergum and sternum 7. Flagellum light ferruginous. Apex of mandible and legs dark brown. Wings dark yellow to ferruginous, darker apically.

TYPE MATERIAL. I have not been able to locate Latreille's types of *lecheguana.* Buysson (1905) indicated that they were in the Paris Museum and had the following labels: "Rio Grande, ouest de la Capitainerie des Mines, Nord de la Capitainerie de Saint-Paul, sud de la Capitainerie de Goyaz." They were collected by A. de Saint Hilaire in 1815 and 1820 at the same time that the types of *augusti* were collected.

Spinola's types of *Brachygastra velutina* have been compared with specimens of *lecheguana* and do not differ significantly. His species has often been considered a variety of *lecheguana* on the basis of the "corps velouté," but I have found the velvety appearance to be striking only in relatively newly emerged wasps in which there was no wing wear. Specimens with worn wings have hairs much shorter and consequently a slightly different appearance.

A male "type" of *Nectarinia binotata* Saussure is in the Paris Museum but it has a different label ("Colombie, C. Parzudacki, 1840") than Saussure's original indication ("Cayenne"). Although the genitalia have not been examined it agrees closely with other males of *lecheguana.*

VARIATION. The characters that vary in this species are the same as those in *mellifica*, but the range of variation is much greater. The form of the scutellum ranges from a strongly emarginate posterior margin (Fig. 12), to the most common condition of *mellifica, i.e.* a straight posterior margin. Separation of these forms, therefore, may be difficult unless males are available. The shape of the scutellum does not vary on a geographical basis only; it is often possible to find several situations at one locality. The most common condition in South America is a distinct, but not deep, V-shaped emargination of the posterior margin of the scutellum, but in Argentina and Paraguay there is a high percentage of specimens with a straight posterior margin. Many specimens from northern Bolivia and the western Amazon Basin have a deep emargination.

In Panama and northwestern South America the *lecheguana* population is strikingly different and deserves special consideration here. In central Panama, almost all *lecheguana* specimens have a distinctly emarginate scutellum which is also narrowed posteriorly giving the structure a biangulate appearance (Fig. 12, 14). The structure is also relatively higher than most specimens of *lecheguana* in South America and *mellifica* in Central America (Fig. 10). In addition, one male* examined had a very wide spiculum and somewhat different proportions of the digital lobe and the parameres (Figs. 19-21). Four additional males, however, show that these characters vary somewhat and may not be indicative of a distinct population. Females, although distinct in central Panama, are not always readily separable from *mellifica* to the North and appear to grade into the condition of the South American forms in eastern Colombia and Venezuela. In western Colombia and Ecuador, however, they are distinct from populations east of the Andes. I do not feel justified at present in separating this population without the examination of more males. I have included it in *lecheguana* because of the similarities of the male genitalia, but as noted above, females very much resemble southern forms of *mellifica*.

The width of the abdominal bands does not vary noticeably, but the maculations on the head and thorax vary in size. The majority of the wasps lack the latter markings entirely, but some specimens in Panama have yellow on the axillar ridges and the dorsal surface of the scutellum. In Argentina many wasps have dense punctures and long hairs similar to those in *borellii*.

DISTRIBUTION. *Brachygastra lecheguana* is very common in much of South America south to Buenos Aires but not south of Ecuador west of the Andes. It appears to be a characteristic of drier forests and open savannas where its nests are conspicuous in high trees. It may also occur in more humid, forested areas but is never as abundant in these habitats.

Specimens have been examined from the following localities: *Argentina*. Prov. Buenos Aires: Buenos Aires; (Delta de Buenos Aires; Eseiza; Gen. Pacheco); San Isidro; (Tigre). Prov. Catamarca: (El Cavillo); La Merced, Prov. Chaco; Resistencia; (Río Ducle). Prov. Cordolsa: Capilla del Monte; Córdoba; Cosquín; Cruz del Eje; (La Bahamondes). Prov. Formosa: (Espinillo; Gran Guardia; Laguna de Blanca; Tres Isletas). Prov. Jujuy: (Dique la Cienaga); Jujuy. Prov. La Rioja. Prov. Missiones: (Loreto); Obera; Río Iguazú. Prov. Salta: (Cabeza de Buey; Potrero de Linares; Río Blanco); Salta; San Bernardo. Prov. Santa Fé: (La Gallereta); Reconquista; Villa Ana; Villa Guillermina. Prov. Santiago del Estero: Colonia Jaime. Prov. Tucumán: Tafi Viejo; Trancas; Tucumán. *Bolivia*. Dept. Beni: (Ivoń); Tumupasa. Dept. Santa Cruz: (Prov. del Sara); Roboré. *Brazil*. Est. Amazonas: Manaus. Est. Ceará: Quixeramobim. Est. Goiás: Anápolis; 5 mi. E. of E. branch Rio Araguaia between Loroti and Rio Formosa; Jatai; Santa Isabel. Est. Guaporé: Pôrto Velho. Est. Mato Grosso: Chapada; Pôrto Velho; Salobra; Utiariti on Rio Papagaio. Est. Minas Gerais: Pouso Alegra. Est. Nova Teutonia: Cauna; (Pinhal); Santa Catarina. Est. Pará: Belém; Lower Rio Liberdade. Est. Paraíba: Mun. Soledade, Joazeirhinho. Est. Paraná: Curitiba. Est. Pernambuco: Bonito. Terr. Roraima [= Terr. Rio Branco]: Carmo (Island); Santa Maria; Vista Alegre. Est. São Paulo: Barretos; Baueri; Batatais; Bauru; Campos do Jordão; (Eng. Cesar de Souza); Faz. do Bonito, Serra da Bocaina; (Fas. Pau d'Alho-Itú); Ipiranga; Monte Alegre, 750 m; Santa Amara (Island); São Carlos, São Paulo. *British Guiana*. Essequibo Co.: (Onderneeming); Rio Essequibo. *Colombia*. Dept. Antioquía; Puerto Berrio. Dept. Atlantico: Puerto Colombia. Dept. Bolivar: Cartagena. Dept. Huila: Villavieja. Dept. Magdalena: Atlantico 200 m; Barranquilla; Cienaga; Río Frio; Santa Marta. Dept. Meta: Restrepo, 500 m; Villavicencio. Dept. Santander: (Boca del Rosaria). Dept. Tolima: 11 mi. E. Ibaque. *Costa Rica*. Alajuela Prov.: Orotina. Puntarenas Prov.: Palmar. *Ecuador*. Prov. Quayas: Quayaquil; 3 mi. N. Manglar, Alto Guayas.

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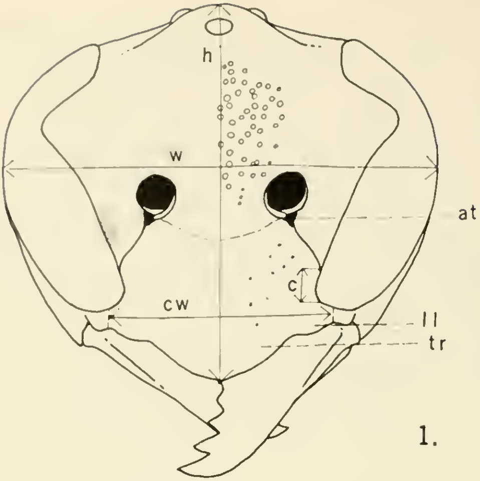
* In the American Museum of Natural History.



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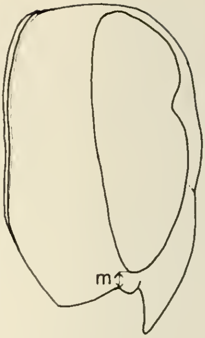

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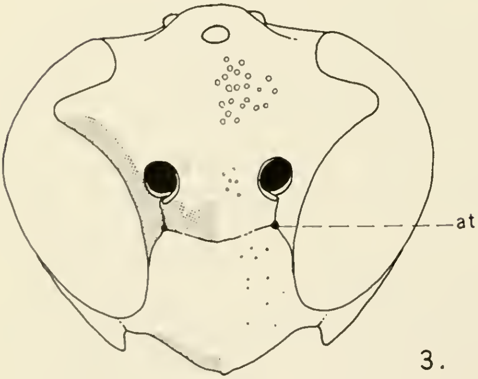

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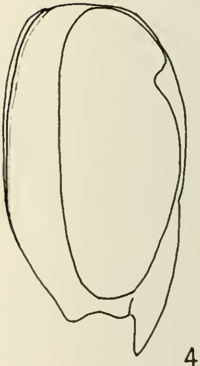

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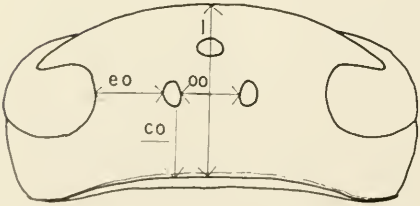

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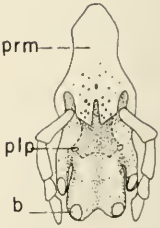

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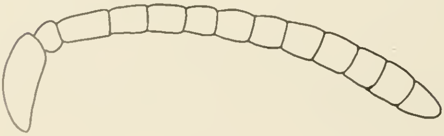

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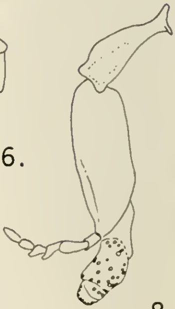

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French Guiana. Dist. Guayane: Cayenne. *Panama.* Canal Zone: Ancón; Barro Colorado Island; Corozal; Ft. Clayton; Gamboa; Juan Mina. Prov. Chiriquí: Boquete; Potrerillos. Prov. Colón: Santa Rosa. Prov. Coclé: Penonome; Calhajuelo. Prov. Panama: Panama; Sabanas. *Paraguay.* Dept. Alto Paraná; Puerto Bertoni. Dept. Caagazú: Tacuara. Dept. Guairá: Villarica. Dept. La Cordillera: San Bernadino. Dept. San Pedro: Independencia; (Paso Yobay). *Peru.* Dept. Cajamarca: Pacasmayo. Dept. Loreto: Río Putumayo, near Taona; Río Ucayali, Middle. Dept. Piura: Queb. Tamarindo, Anotape Mts. Dept. Tumbes: Tumbes. (San Antonio on Río Cotuhe; Rio Cliotano; Yeguestesque). *Surinam.* Dist. Commewijne: Matapica. Dist. Marowijne: Albina; Moengatapoe. Dist. Suriname: Braamspunt; Kwakoegron; Paramaribo. (Boskamp; Lelydorp). *Venezuela.* Est. Barinas: Barinas; San Silvestre. Est. Bolívar: Cuidad Bolívar; Suapure on Río Caura. Terr. Delta Amacuro: 140 km. N.E. Barrancas on Caño Mariusa. Dist. Federal: Caracas. Est. Trujillo: Valera. Est. Zulia; Encontrados; Río Negro.

BIOLOGY. Owing to its interesting habit of storing large amounts of honey in its nest, this species was noted in the literature as early as 1648. Early accounts of honey storing bees and wasps undoubtedly refer in part to *B. lecheguana.* Bequaert (1932) gives an excellent history of the knowledge of this species and the reader is referred to his work for a more detailed account of the reference to *lechguana.*

The nest is like that of *mellifica* and may become very large. R. von Ihering (1904) described a nest from São Paulo consisting of 20 combs and measuring 27 by 39 cm. According to Bertoni (1912) the nest is very common in Paraguay and can be found low in the underbrush and grasses where it may reach a diameter of 30 cm. It is generally spherical to elongate and has several entrances. The internal structure is phragmocyttarous like *mellifica*, and the cells are 4-5 mm. wide and 6-7 mm. deep. The full grown larva spins a cap 4-5 mm. high over this cell.

The colonies are founded by swarms and are perennial. The wasps are able to withstand low temperatures (–5° C.)and, according to Wagner, retreat into the cells with only the tips of the abdomens protruding during the winter.

Brachygastra borellii (Zavattari)
(Fig. 65)

Caba borellii Zavattari, 1906. Boll. Mus. Anat. Comp. Torino 21(523):1.
Nectarina lecheguana var. *borellii,* Ducke, 1910. Ann. Mus. Nat. Hungarici 8:482.
Brachygastra lecheguana var. *borelli,* Willink, 1952. Acta. Zool. Lillolana (Tucumán) 10:146.

FIG. 1. Frontal view of head of female *B. lecheguana.* at=anterior tentorial pit; c=contact with eye; cw=width of clypeus; h=height of head; ll=lateral lobe of clypeus; tr=apical triangle of clypeus; w=width of head.

FIG. 2. Lateral view of head of female *B. lecheguana.* m=malar space.

FIG. 3. Frontal view of head of *B. lecheguana.* at=anterior tentorial pit.

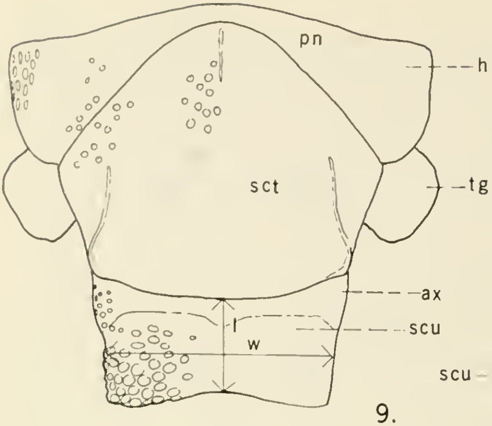
FIG. 4. Lateral view of head of male *B. lecheguana.*

FIG. 5. Dorsal view of head of female *B. lecheguana.* eo=distance between eye and lateral ocellus; co=distance between occipital carina and lateral ocellus; l=length of head; oo=distance between lateral ocelli.

FIG. 6. Caudal view of labium of *B. lecheguana.* b=acroglossal button; plp=posterior lingual plate; prm=prementum.

FIG. 7. Antenna of male *B. lecheguana.*

FIG. 8. Caudal view of right maxilla of *B. lecheguana.*



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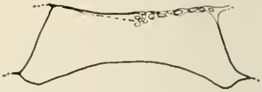

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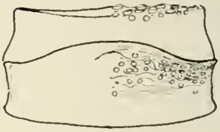

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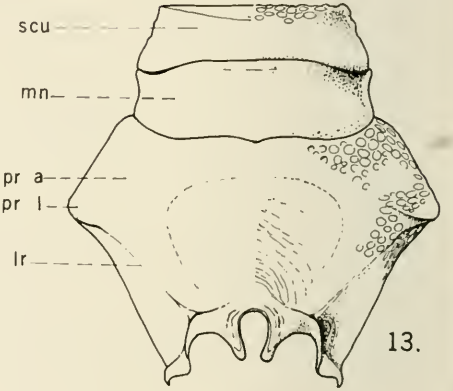

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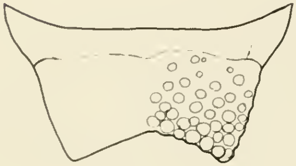

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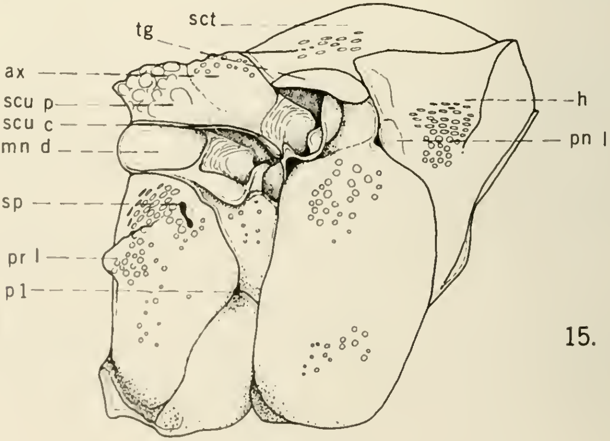

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Because *borellii* was described from a single female and is not in most collections, both Ducke and Bequaert thought it to be an "aberration" of *lecheguana*. Willink (1952), after examining two additional specimens from northern Argentina, concluded that Zavattari's species was a variety of *lecheguana*. Additional material from Bolivia and Argentina seems to indicate that it has a much wider range than originally expected and that it may, in fact, be a distinct species. Although it is similar to *lecheguana* with respect to most characters, *borellii* is quite unique on the basis of pubescence and punctations.

Brachygastra borellii is best distinguished from *lecheguana* by the very long, yellow hairs and the dense, deep punctures on the head and thorax. It is an entirely black wasp, the yellow markings only faintly appearing on the apical margin of the second tergum. The wings are dark and relatively long, extending a considerable distance beyond the abdomen. In addition, the dense, punctured, rugose surface of the metanotum is unique and will separate *borellii* from other species (Fig. 11).

FEMALE. (1.) Wing length 7.98 mm.

Head. (2.) In frontal view .88 times as high as wide; in dorsal view about .42 times as wide as long; posterior margin slightly curved. (3.) Lateral ocellus separated from eye by 1.45 distance between lateral ocelli and from occipital carina by 1.41 this distance; vertex with medium sized deep punctures separated by less than one diameter, usually contiguous; vertex strongly convex, posterior surface sloping ventrad in profile. (4.) In lateral view gena about 1.3 times as wide as eye in middle; postgenal convexity absent, ventral half of posterior margin of gena straight, gena widest at middle; gena about as wide on ventral half as at level of eye emargination; punctures medium sized, separated by about one to two diameters, evenly spaced on entire gena. (5.) Occipital carina forming an acute ridge about 0.3 times as high as width of ocellus, slightly lower medially, extending to mandibular condyle. (6.) Frons with dense, contiguous, medium sized punctures. (7.) Clypeus about

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FIG. 9. Dorsal view of thorax of *B. lecheguana*. ax=axilla; h=humeral angle; l=length of scutellum; pn=pronotum; sct=scutum; scu=scutellum; tg=tegula; w=width of scutellum.

FIG. 10. Caudal view of scutellum of *B. lechequana*, Panama Canal Zone.

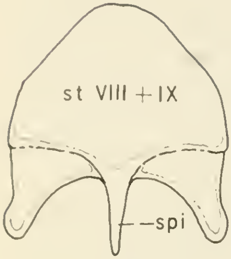
FIG. 11. Caudal view of scutellum and metanotum of *B. borellii*.

FIG. 12. Dorsal view of scutellum of *B. lecheguana*, Panama, Canal Zone.

FIG. 13. Caudal view of thorax of *B. lecheguana*. lr=lateral ridge of propodeum; mn=metanotum; pr a=propodeal angle; pr l=propodeal lobe; scu=scutellum.

FIG. 14. Lateral view of scutellum of *B. lecheguana*, Panama, Canal Zone.

FIG. 15. Lateral view of thorax of *B. lecheguana*. ax=axilla; h=humeral angle; mn d=metanotal depression; p l=first metapleural pit; pn l=pronotal lobe; pr l=propodeal lobe; sct=scutum; scu c=scutellar crest; scu p=scutellar pocket; tg=tegula.



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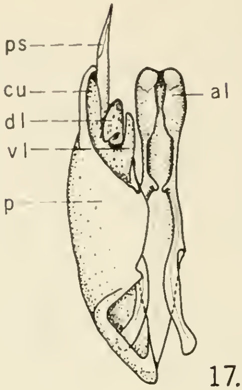

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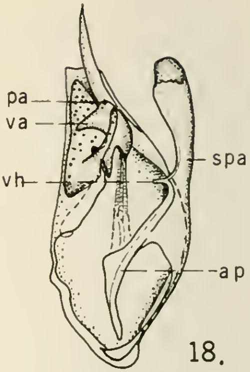

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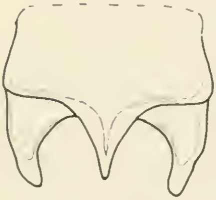

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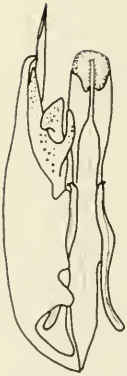

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1.7 times as wide as long, slightly convex; distal margins curved, broadly rounded onto lateral lobes; apical triangle short, about 0.8 times as long as width of antennal socket, apex broadly rounded; contact with eye equal to about 0.8 width of antennal socket; epistomal suture forming about a 45° angle with eye margin, dorsally, scarcely evident, broadly curved ventrad; clypeal surface occasionally irregular, somewhat shiny, with small punctures separated by about two diameters basally, larger, almost contiguous apically, almost entirely sericeous. (8.) Malar space about 0.4 width of antennal socket. (9.) Antenna with flagellum moderately swollen, eighth flagellomere about 1.5 times as wide as long. (10.) Head with abundant, long, erect, golden hairs little more than twice as long as width of ocellus, longer on vertex than elsewhere; eye with abundant hairs about as long as width of ocellus; head very lightly sericeous.

Thorax. (11.) Anterior surface of pronotum with few scattered punctures dorsally, distinctly separated from dorsal surface of pronotum; pronotal keel low, of even height, acute, extending to humeral angle only, absent on lateral surface; humeral angle not well developed, rounded; dorsal surface abruptly rounded onto lateral surface; lateral surface not distinctly separated from anterior surface, with medium sized punctures separated by about one diameter; pronotal lobe wide, distinct. (12.) Scutum about 0.9 times as long as wide, with evenly spaced, medium sized punctures separated by one to two diameters. (13.) Scutellum in dorsal view about twice as wide as long, slightly bilobed, only slightly shorter at middle than at lateral margin, posterior margin forming a slight V; in posterior view, about 5 times as wide as high at middle, dorsal margin almost straight, slightly depressed medially; in profile, scutellum angular, not raised above plane of scutum, extending slightly over plane of metanotum; dorsal surface flat to slightly convex,

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FIG. 16. Dorsal view of sternum VIII + IX and spiculum of *B. lecheguana*. spi=spiculum.

FIG. 17. Ventral view of aedeagus and left paramere of *B. lecheguana*. al=aedeagal lobe; cu=cuspis; dl=digital lobe; p=paramere; ps=parameral spine; vl=volsellar lobe.

FIG. 18. Mesal view of aedeagus and left paramere of *B. lecheguana* (aedeagus displaced dorsad). ap=aedeagal apodeme; pa=posterior angle of digital lobe; spa=spatha; va=ventral angle of digital lobe; vh=ventral hook.

FIG. 19. Dorsal view of base of sternum VIII + IX and spiculum of *B. lecheguana*, Panama, Canal Zone.

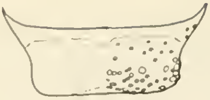
FIG. 20. Ventral view of aedeagus and left paramere of *B. lecheguana*, Panama, Canal Zone.

FIG. 21. Mesal view of aedeagus and left paramere of *B. lecheguana*, Panama, Canal Zone.

FIG. 22. Dorsal view of base of sternum VIII + IX and spiculum of *B. mellifica*.

FIG. 23. Ventral view of aedeagus and left paramere of *B. mellifica*.

FIG. 24. Mesal view of aedeagus and left paramere of *B. mellifica*.



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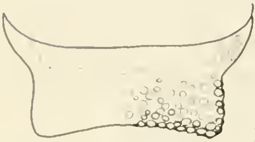

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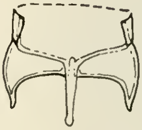

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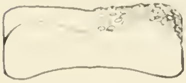

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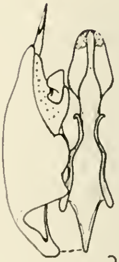

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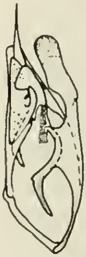

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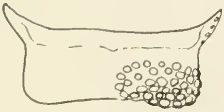

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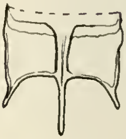

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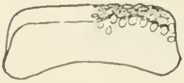

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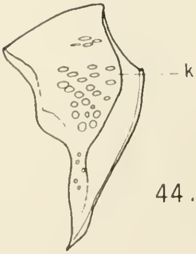

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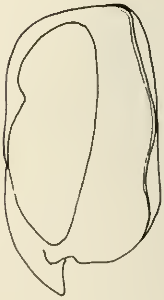

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posterior surface flat; scutellar pocket flat, with large contiguous punctures; dorsal surface with large, shallow, contiguous punctures, posterior surface with scattered, medium sized punctures dorsally; axillar ridge very wide, flattened, with small punctures. (14.) Metanotum about 3 times as wide as long, convex; dorsal margin bowed evenly dorsad, ventral margin bowed weakly ventrad; surface punctured, irregularly rugose. (15.) Mesopleuron moderately convex; anterior and posterior surfaces with scattered small punctures; punctures large, shallow, contiguous, forming rugose sculpturing dorsally, punctures medium sized separated by about one diameter ventrally. (16.) Dorsal sclerite of metapleuron twice as high as width at middle, with many small punctures; secondary suture distinct, forming right angle with intersegmental suture; first metapleural pit shallow; ventral sclerite with small punctures separated by one to three diameters; metapleural-propodeal suture evident as a wide, shallow furrow. (17.) Propodeum angular; posterior surface with narrow, deep, median concavity, median area with weak irregular horizontal striations, dorsolateral area with large, shallow, contiguous punctures forming rugose sculpturing; lateral surface with medium

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Fig. 25. Dorsal view of scutellum of *B. azteca.*

Fig. 26. Lateral view of scutellum of *B. azteca.*

Fig. 27. Caudal view of scutellum of *B. azteca.*

Fig. 28. Lateral view of propodeum of *B. azteca.*

Fig. 29. Ventral view of aedeagus and left paramere of *B. azteca.*

Fig. 30. Mesal view of aedeagus and left paramere of *B. azteca.*

Fig. 31. Dorsal view of scutellum of *B. fistulosa.*

Fig. 32. Lateral view of scutellum of *B. fistulosa.*

Fig. 33. Dorsal view of base of sternum VIII + IX and spiculum of *B. azteca.*

Fig. 34. Caudal view of scutellum of *B. fistulosa.*

Fig. 35. Caudal view of scutellum of *B. augusti.*

Fig. 36. Lateral view of scutellum of *B. augusti.*

Fig. 37. Ventral view of aedeagus and left paramere of *B. augusti.*

Fig. 38. Mesal view of aedeagus and left paramere of *B. augusti.*

Fig. 39. Dorsal view of scutellum of *B. scutellaris.*

Fig. 40. Lateral view of pronotum of *B. augusti.*

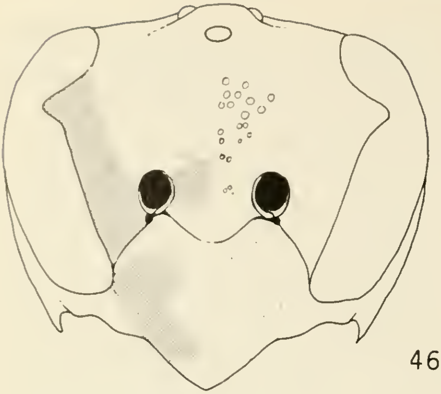
Fig. 41. Dorsal view of base of sternum VIII + IX and spiculum of *B. augusti.*

Fig. 42. Caudal view of scutellum of *B. scutellaris.*

Fig. 43. Lateral view of scutellum of *B. scutellaris.*

Fig. 44. Lateral view of pronotum of *B. scutellaris.* k=pronotal keel.

Fig. 45. Lateral view of head of female *B. scutellaris.*



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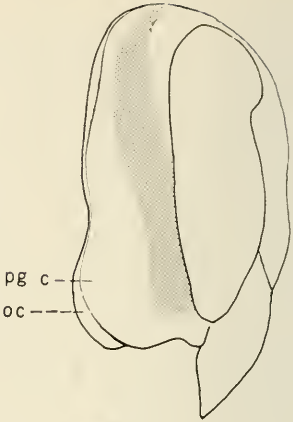

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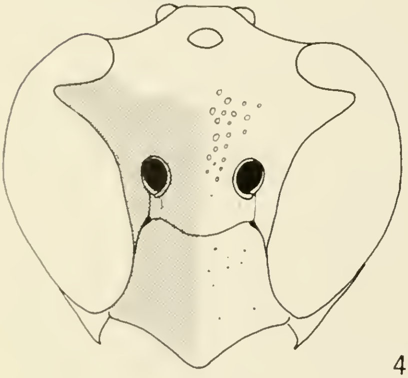

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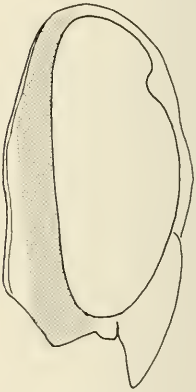

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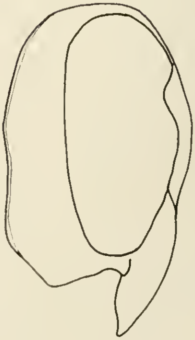

51.

to large sized punctures separated by one diameter or less, smaller anteriorly and ventrally, rugose posteroventrally; lateral ridge incomplete, present only on propodeal angle; propodeal angle with compressed, blade-like rounded projection, forming prominent round lobe in lateral view, an abruptly truncate, lateral shelf-like ridge in posterior view. (18.) Thorax with abundant long yellow hairs, lightly sericeous.

Abdomen. (19.) Tergum 1 wide, cap-shaped not distinctly set off from tergum 2, about 4.4 times as wide as long; sternum 1 about 4 times as wide as long; tergum with scattered small punctures, sternum rugulose. (20.) Tergum 2 about .86 times as long as wide, low evenly convex in profile; tergum 2 with evenly spaced small punctures separated by about two diameters, sternum 2 with punctures more widely spaced. (21.) Terga and sterna 2-5 with punctures as on 2; tergum and sternum 6 with few small punctures. (22.) Abdomen with abundant yellow hairs directed caudad, about as long as width of ocellus; abdomen lightly sericeous.

Coloration. Almost entirely black, occasionally with very narrow, incomplete yellow apical band on tergum 2. Apex of mandible and tarsi dark brown. Wings brown, veins dark brown.

TYPE MATERIAL. The type, a female from Salta, Argentina, was probably deposited in the Instituto e Museo di Zoologia in Torino, Italy, but it cannot be found there. It is possible that it was destroyed during the war.

VARIATION. Specimens from Bolivia have both humeral and propodeal angles slightly less developed than specimens from Argentina. The propodeal angle is especially well developed in the Argentine specimens and this was the condition in the type; "margini del metatorace compressi formanti un angoli spiniforme acuto ben distincto."

The width and extent of the apical band on the second abdominal segment is quite variable both in Argentina and Bolivia although it is never well developed.

DISTRIBUTION. Although described from Salta, Argentina, additional material from Bolivia indicates that it has a much wider range. It appears to replace *baccalaurea* in the southern Andes as both species are apparently high altitude forms and have not been found together. It is also interesting to

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FIG. 46. Frontal view of head of female *B. smithii.*

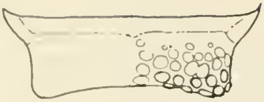
FIG. 47. Lateral view of head of female *B. smithii.* pg c=postgenal convexity; oc=occipital carina.

FIG. 48. Frontal view of head of male *B. smithii.*

FIG. 49. Lateral view of head of male *B. smithii.*

FIG. 50. Lateral view of head of female *B. buyssoni.*

FIG. 51. Lateral view of head of female *B. bilineolata.*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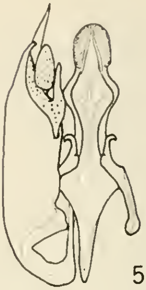
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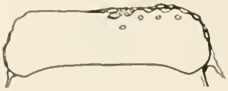
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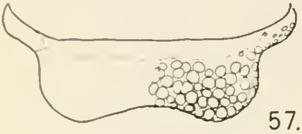
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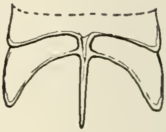
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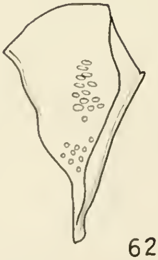
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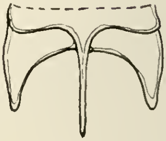
67.

FIG. 52. Dorsal view of scutellum of *B. bilineolata.*

FIG. 53. Lateral view of scutellum of *B. bilineolata.*

FIG. 54. Caudal view of scutellum of *B. bilineolata.*

FIG. 55. Ventral view of aedeagus and left paramere of *B. bilineolata.*

note that both wasps have the extremely melanic pigmentation and the well developed pubescence often characteristic of high altitude species.

I have seen specimens from the following localities: *Argentina.* Salta Prov.: Abra Santa Laura. Jujuy Prov.: Lagunas de Yala. *Bolivia.* Dept. Cochabamba: Cochabamba, 2,600 m.

Schrottky (1913) reported *borellii* from Tucumán, Argentina, about 140 mi. southwest of the type locality.

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FIG. 56. Mesal view of aedeagus and left paramere of *B. bilineolata.*

FIG. 57. Dorsal view of scutellum of *B. smithii.*

FIG. 58. Lateral view of scutellum of *B. smithii.*

FIG. 59. Dorsal view of base of sternum VIII + IX and spiculum of *B. bilineolata.*

FIG. 60. Caudal view of scutellum of *B. smithii.*

FIG. 61. Lateral view of pronotum of *B. baccalaurea.*

FIG. 62. Lateral view of pronotum of *B. smithii.*

FIG. 63. Ventral view of aedeagus and left paramere of *B. smithii.*

FIG. 64. Mesal view of aedeagus and left paramere of *B. smithii.*

FIG. 65. Caudal view of scutellum and metanotum of *B. baccalaurea.*

FIG. 66. Lateral view of propodeum of *B. buyssoni.*

FIG. 67. Dorsal view of base of sternum VIII + IX and spiculum of *B. smithii.*

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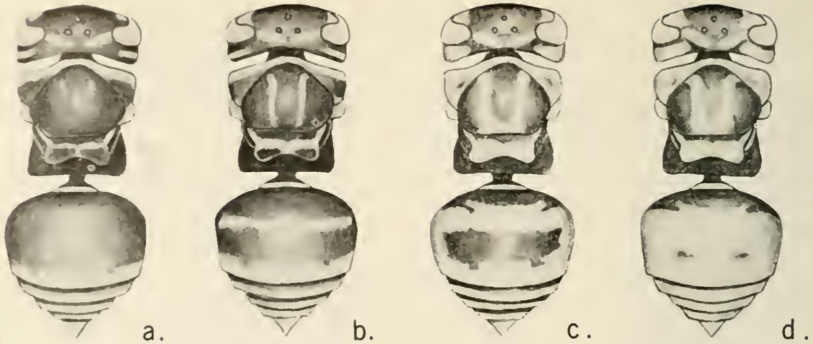

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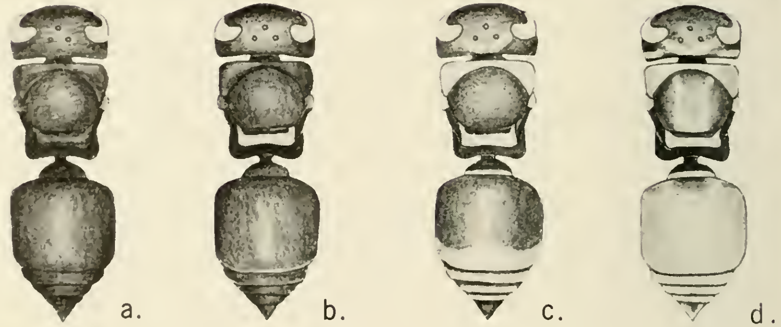

Fig. 68. Color variation in *B. bilineolata* in northern Surinam. a. Paramaribo; b. Blauwgrond; c. Republick; d. Zanderij.

Fig. 69. Color variation in *B. scutellaris* in Colombia. a-b. Restrepo, Dept. Meta.; c-d. Muzo, Dept. Boyaca.

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THE UNIVERSITY OF KANSAS
SCIENCE BULLETIN

HYBRIDIZATION AND CLASSIFICATION OF *HAPLOPAPPUS BRICKELLIOIDES*

By

R. C. Jackson

Vol. XLVII Pages 1005-1012 March 26, 1968 No. 18

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THE UNIVERSITY OF KANSAS
SCIENCE BULLETIN

Vol. XLVII Pages 1005-1012 March 26, 1968 No. 18

Hybridization and Classification of *Haplopappus brickellioides*[1]

R. C. Jackson
Botany Department

In his monographic treatment of *Haplopappus*, Hall (1928) classified *Haplopappus brickellioides* Blake as a member of the section *Blepharodon* and suggested that its scarcity and wide morphological divergence indicated that it might be a relict from some other extinct branch of the section. *Haplopappus brickellioides* does indeed appear to be narrowly restricted in its distribution. Apparently it occurs only on limestone outcroppings in Inyo County, California, and Nye and Clark counties of Nevada. However, in Clark County at least it is locally abundant on the south slopes in the southern part of the Spotted Range.

In the original species description and in that given by Hall (1929), *H. brickellioides* was described as eradiate. However, I have carefully examined the holotype (US) and isotype (UC) and found that ray flowers were present but not well developed in the immature heads. Short but well developed rays were found subsequently on all other specimens examined.

MATERIALS AND METHODS

Seeds and specimens of *H. brickellioides* were obtained several years ago as part of a biosystematic investigation of section *Blepharodon*. Seeds were germinated in small flasks containing tap water which was changed several times. Some of the seedlings were transferred to soil in pots in the greenhouse after the hypocotyl had elongated 5 to 10 mm.

Chromosome counts were obtained from root tip cells and microsporocyte squashes by techniques described previously (Jackson, 1965). Fertility

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[1] This study was supported by NSF Grant GB-3071.

was determined by counting the number of pollen grains with a cytoplasm fully stained by lactophenol and cotton blue in a sample size of over 500 grains from each plant.

Chiasma frequency in the F_1 hybrids was determined by calculating the minimum number necessary to form a particular configuration. In the parental plants, chiasmata were determined directly as there was no difficulty in analyzing the bivalents. Even where only bivalents are concerned, however, there is always the possibility that some chiasmata have terminalized by diakinesis so that one is essentially reading the minimum number for a particular configuration.

Sources of the parental plants used in the successful crosses are as follows: *Haplopappus brickellioides* Blake, Clark County, Nevada, about 4 miles north of Logandale, Spotted Range, 23 Oct. 1963, *Johnson 2117* (KANU); *H. squarrosus* Hook. & Arn. subsp. *squarrosus*, San Luis Obispo County, California, 24 Sept. 1960, *Wells sn.* (KANU). A specimen of the F_1 hybrid of these two is also deposited at KANU.

ATTEMPTED HYBRIDIZATIONS

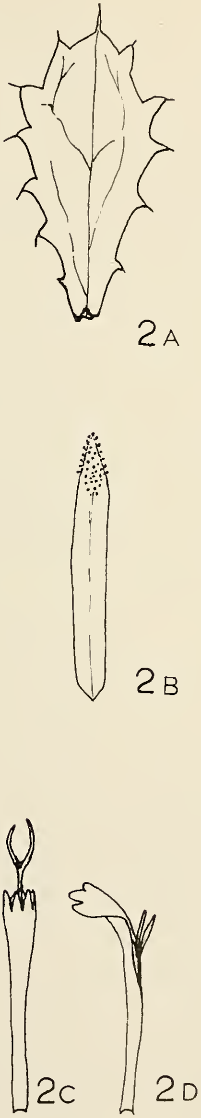
Crosses were attempted between *H. brickellioides* and several species of section *Blepharodon*, namely, *H. arenarius* Benth. subsp. *arenarius* and var. *incisifolius* Johnst., *H. texensis* Jackson, and several subspecific taxa of *H. spinulosus* Pursh. The results of all such crosses were negative; no mature achenes were obtained.

Hybridizations were attempted also between *H. brickellioides* and *H. venetus* subsp. *vernonioides* (Nutt.) Hall of section *Isocoma* and between *H. brickellioides* and *H. squarrosus* Hook. & Arn. subsp. *squarrosus* of section *Hazardia*. Only the latter cross was successful, and three F_1 hybrids were grown to maturity in the greenhouse.

MORPHOLOGY OF F_1 HYBRIDS

Comparative morphology of *Haplopappus brickellioides* × *H. squarrosus* Hook. & Arn. subsp. *squarrosus* shows the F_1 as generally intermediate for vegetative and floral characters of the parental plants (Figs. 1-3). An apparent exception to this is the well developed ligule of the ray flowers in the F_1 (Fig. 2D).

The two parental taxa certainly have more features in common than either has with any species of section *Blepharodon*. Both species have thin, leathery leaves typical of the Mediterranean type of vegetation found in southern and coastal California. Their leaves are sharply serrate with mucronate teeth but different in size. Both have involucres that are turbinate to



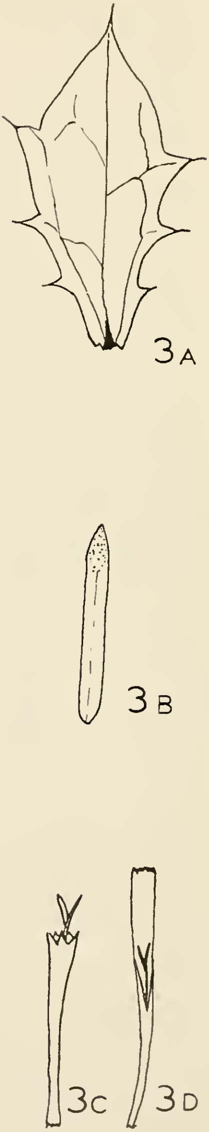


FIG. 1. *Haplopappus squarrosus* subsp. *squarrosus:* 1A, median leaf. 1B, inner phyllary. 1C, disc flower corolla.

FIG. 2. *H. squarrosus* subsp. *squarrosus* × *H. brickellioides,* artificial F_1 hybrid: 2A, median leaf. 2B, inner phyllary. 2C, disc flower corolla. 2D, ray flower corolla.

FIG. 3. *H. brickellioides:* 3A, median leaf. 3B, inner phyllary. 3C, disc flower corolla. 3D, ray flower corolla.

Magnifications for Figs. 1A and 2A is ×1, Fig. 3A is ×2. Figs. 1B to 3D are ×4.

FIG. 4. Diakinasis in *Haplopappus squarrosus* subsp. *squarrosus* showing five bivalents.
FIG. 5. Diakinesis in *H. brickellioides* showing six bivalents.

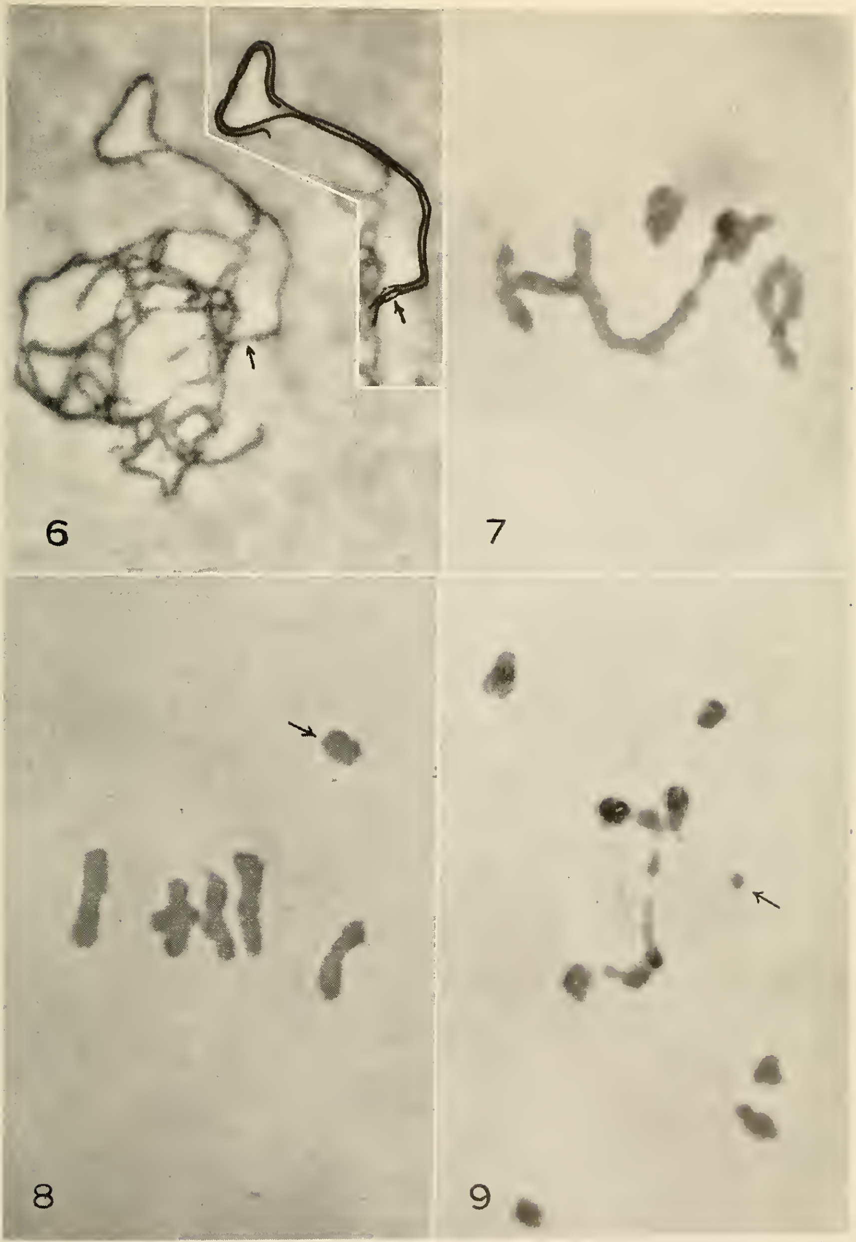
subcylindric with somewhat squarrose bracts. In all evident morphological characters, *H. brickellioides* is usually smaller than *H. squarrosus* subsp. *squarrosus*.

CYTOLOGY OF F_1 HYBRID

The chromosome number of *Haplopappus squarrosus* subsp. *squarrosus* is $n = 5$ (Fig. 4) as first reported by De Jong and Montgomery (1963). *H. brickellioides* is $2n = 12$, $n = 6$ (Jackson, 1966), as determined from both root tip cells and microsporocytes (Fig. 5). The mean minimum chiasma frequency was determined for both species at diakinesis. *H. brickellioides* had a mean minimum chiasma frequency of 9.5 per cell in 33 cells with a range of 8 to 11. The mean minimum chiasma frequency of 31 cells of *H. squarrosus* subsp. *squarrosus* was 5.9 with a range of 5 to 7. These data are from only the two plants used in the production of the F_1 interspecific hybrids. Pollen fertility of both parental plants ranged from about 97 to 100 percent.

Meiosis was studied in some detail in the three F_1 hybrids. Because no morphological or cytological differences were noted among the hybrids, the diakinesis pairing data in Table 1 is a compilation from the three F_1 plants.

Pachytene stages were difficult to interpret, but complex translocation configurations were noted. A typical inversion configuration (Fig. 6) was observed in several cells.



Figs. 6 to 9. Meiotic stages in the artificial F_1 hybrid *Haplopappus squarrosus* subsp. *squarrosus* × *H. brickellioides.*

Fig. 6. Pachytene showing paracentric inversion configuration; diagrammatic representation is shown in the inset. An arrow shows position of the centromeres.

Fig. 7. Diakinesis showing a bivalent, a trivalent, and a hexavalent.

Fig. 8. Metaphase I showing five bivalents and a univalent (arrow). Note that bivalents are generally heteromorphic.

Fig. 9. Anaphase I showing a trivalent association in which a dicentric bridge connects two of the chromosomes. A fragment is indicated by the arrow.

Pairing configurations in the F_1's were analyzed and scored at diakinesis, and the mean minimum chiasma frequency was obtained from these data (Table 1). A number of complex multivalent arrangements were observed. Over half of the 34 cells analyzed had at least a quadrivalent at diakinesis or metaphase I, and there was a range of configurations from three bivalents and five univalents to a bivalent, a trivalent, and a hexavalent (Fig. 7). One of the 34 cells analyzed had five bivalents and a univalent (Fig. 8).

A dicentric bridge and a fragment were noted in several cells at anaphase I (Fig. 9), indicating that the inversion configuration noted at pachytene (Fig. 6) was of the paracentric type. Observed distributions to the poles at A_1 were $4 - 4 + 2$ laggards, 5-6, 4-6, and 8-3.

Pollen fertility, as determined by stainability, was 3.4 percent; 37 pollen grains of 1073 were stainable, and most of these appeared to be reconstituted mother cells as judged by their large size. This is contrasted to the 97-100 percent fertility of the parent plants.

DISCUSSION AND CONCLUSIONS

There is no basis on morphological grounds for classifying *Haplopappus brickellioides* Blake as a member of section *Blepharodon*. It is easily separable from *Blepharodon* by its turbinate to cylindric involucre although Hall (1929) refers to it as campanulate, probably because the material he examined had immature heads. If the involucre is correctly characterized,

Table 1. Chromosome pairing configurations, their frequency, and minimum chiasmata number in the F_1 hybrid *Haplopappus brickellioides* × *H. squarrosus* subsp. *squarrosus*.

Number of cells	Configuration	Minimum chiasmata
1	II,III,VI	8
4	I,2II,VI	7
1	II,IV,V	8
1	3II,V	7
1	I,II,III,V	7
2	2I,2II,V	6
1	2I,IV,V	7
3	2II,III,IV	7
1	I,II,2IV	7
2	2I,II,III,IV	6
5	I,3II,IV	6
1	3I,2II,IV	5
1	II,3III	7
3	I,2II,2III	6
4	2I,3II,III	5
2	I,5II	5
1	5I,3II	3
34	Totals	210
		$\overline{X} = 6.17$

H. brickellioids can be keyed easily to section *Hazardia* in Hall's monograph, and I am classifying it as a member of this section because of its close morphological similarity to other species of the taxon and because of its crossing relationship with *H. squarrosus* subsp. *squarrosus*.

Pairing at pachytene in the F_1 hybrid *H. brickellioides* × *H. squarrosus* subsp. *squarrosus* indicated that rather long chromosome segments of the parents were homologous, and the high chiasmata frequency attests to this also. No deletion or duplication configurations were noted. With the exception of the paracentric inversion observed, the primary cause of sterility in the F_1 hybrids can be attributed to reciprocal translocations among nonhomologous chromosomes that have occurred in one or both species after or during the time of their divergence. Since data are not available on karyotype divergence in the species of *Hazardia*, no comparative analysis can be attempted with *H. brickellioides* at this time.

Cytologically, *H. brickellioides* appears to be an anomaly in section *Hazardia*. Species of this section for which chromosome counts of $n = 5$ have been reported are *H. squarrosus* subsp. *squarrosus* (De Jong & Montgomery, 1963), *H. canus* (Raven *et al.*, 1960), *H. orcuttii*, and *H. berberoidis* (R. C. Jackson & R. Moran, unpublished). Chromosome numbers for some other sections of *Haplopappus* are $X = 4$ for *Blepharodon* (Jackson, 1962), $X = 4$ or 5 for *Osbertia*, $X = 6$ for *Isocoma*, and $X = 9$ for *Ericameria*, *Stenotus*, and *Macronema*. Under orthodox evolutionary reasoning, the groups with $n = 9$ are generally more primitive morphologically than those with lower numbers. However, where more than one chromosome number is known for a section, the matter of basic number must remain in doubt until cytogenetic evidence determines the direction of chromosome change. The basic (X) chromosome number has been determined by cytogenetic analysis only for section *Blepharodon*.

In *H. brickellioides* the chromosome number is a noticeable exception for the section *Hazardia*, and it is tempting to consider $n = 6$ as an ascending aneuploid on the base of $X = 5$. However, there was no evidence for this during a meiotic analysis of *H. brickellioides*, and the several translocations that have occurred during the evolution of *H. brickellioides* and *H. squarrosus* subsp. *squarrosus* make an interpretation from meiosis in the F_1 hybrid difficult at this time. A knowledge of karyotype evolution of all the species and appropriate hybrids may eventually solve the problem.

In lieu of these data and the ascending aneuploidy hypothesis, I would like to offer at this time the alternative suggestion that $n = 6$ is the more primitive number in section *Hazardia*. *H. brickellioides* is well isolated from other species of the group and could represent a relict population containing the true basic number for the section. With the exception of *H. whitneyi* Gray, for which there is no chromosome count reported, the other taxa of *Hazardia* are closely related morphologically and are probably of relatively recent origin. Several of the species are known to hybridize naturally. The species with $n = 5$ could have evolved from an ancestral aneuploid which had successfully undergone the transition from $n = 6$ to $n = 5$. If Hall's (1928) ideas on the derivation of section *Hazardia* from a line that had previously produced section *Isocoma* are correct, this might be added weight to the line of reasoning presented because chromosome numbers based on $n = 6$ are all that are known from this latter and presumably more primitive group.

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