

# A Quantitative Taxonomic Study of the *Hoplopleura hesperomydis* Complex (Anoplura, Hoplopleuridae), with Notes on *A Posteriori* Taxonomic Characters<sup>1</sup>

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## Abstract

Discrimination of adults of the three closely related species of the *Hoplopleura hesperomydis* complex of lice (Anoplura) is discussed, including a standardized system for taking measurements, followed by various statistical analyses of the data. Certain measurements are shown to have taxonomic value in discriminating between species after analysis, and the method of *a posteriori* selection of such characters is described. The literature on statistical means of species differentiation is reviewed.

## Introduction

The louse species, *Hoplopleura ferrisi* Cook and Beer (1959), was originally differentiated from *Hoplopleura hesperomydis* (Osborn, 1891) *sensu lato* on the basis of characters found solely in the immature stages. The adult stage of *H. ferrisi* was assumed to be identical with that of *H. hesperomydis sensu stricto* in general morphology, at the time of description.

Since 1960, the present authors have carried out various studies on the proper application of statistical methods to taxonomic problems using samples of the species of Hoplopleuridae. Upon studying the *Hoplopleura hesperomydis* complex, the senior author has discovered that it includes several new taxa. All of the taxa, except for *H. reithrodontomydis* Ferris, have usually been mistakenly identified as

*H. hesperomydis* by using Ferris' monograph (1951). Nine taxa (eight species and one subspecies) can be recognized in the *H. hesperomydis* complex (Kim, 1965).

Cook and Beer (1959) studied the immature stages of North American species of *Hoplopleura*, and described two new species of the *H. hesperomydis* complex, *H. ferrisi* from *Peromyscus boylii*, *P. nasutus*, and *P. eremicus*, and *H. onychomydis* from *Onychomys torridus*. *Hoplopleura onychomydis* Cook and Beer is distinct in both adult and nymphal stages from *H. hesperomydis s. s.*, while *H. ferrisi* is distinguished only on the basis of immature stages. This has left *H. hesperomydis s. s.* restricted to *Peromyscus maniculatus*, *P. gossypinus*, *P. californicus*, *P. truei*, and *P. nuttalli*.

The objectives of this study were to differentiate adult stages of *H. ferrisi*, *H. hesperomydis*, and *H. onychomydis*, to demonstrate the use of some statistical procedures in taxonomic problems of specific and intra-specific levels, and to illustrate the investigation of *a posteriori* taxonomic characters for practical use. The term "*a posteriori*" is used in this paper to refer to taxonomic

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characters chosen for use in subsequent identification, once a cryptic species has been recognized. In this paper a standard mensuration system for *Hoplopleura* is discussed, and the data are analyzed using character means, standard deviations, probabilities of misidentification, and discriminant functions for the species. The methods of selecting taxonomic characters are also demonstrated.

The tasks of systematists and the nature of species are discussed, and the literature pertaining to statistical means of recognizing species is reviewed chronologically.

### Materials

Three species of hoplopleurid lice, *H. ferrisi*, *H. hesperomydis*, and *H. onychomydis*, were studied. Specimens used were drawn from the University of Minnesota Entomology Collection. These consisted of 81 males and 102 females of *H. ferrisi* from 66 skins of *Peromyscus boylii* (from Portal, Arizona), 11 of *P. nasutus* (Portal, Arizona), 2 of *P. eremicus* (Wilna, New Mexico); 7 males and 20 females of *H. hesperomydis* collected from several skins of *P. leucopus* (Cedar Creek Forest, Anoka County, Minnesota); 54 males and 57 females of *H. hesperomydis* collected from several hundreds of *P. maniculatus* (Basswood Lake and Rosemount, Minnesota; Beverly Beach State Park, Oregon; Douglas, Wyoming; and Valle, Arizona); and 11 males and 13 females of *H. onychomydis* collected from nine skins of *Onychomys torridus* (Portal, Arizona). All louse specimens were recovered from host skins by Cook's method (1954a, 1954b), and all were given the same treatment and mounted on slides according to a standard procedure.

### Samples and Sampling

Sampling of host specimens was carried out according to procedures described by Cook et al. (1955, 1958) and Beer et al. (1958, 1959).

The number of louse specimens of each species necessary for discrimination was determined on the basis of previous studies

(Kim, 1962; Kim, Brown, and Cook, 1963). Twelve males and 12 females of each species were judged adequate. All microscope slides, each containing a louse specimen, were put in the slide box and assigned consecutive call numbers. Slides for each sex, for the individual species, were drawn from the slide box by calling the number from a table of random numbers (Dixon and Massey, 1957). If the specimen drawn by call number was distorted or partly damaged in any part, an adjacent slide was used. By using this procedure 12 males and 12 females of *H. ferrisi* and *H. hesperomydis* were drawn, but only 11 males and 11 females of *H. onychomydis* were available for this study.

In such a study as this, each sample should include specimens from a number of host animals. A large number of specimens from one or two host animals may not furnish a firm foundation for inference concerning ectoparasite population or species differences (Kim, Brown, and Cook, 1963). If specimens from a number of host animals are used, the data can be subjected to tests of homogeneity. Our earlier studies show that differences may be encountered between louse populations from different localities, host subspecies, host sex, and even different individuals. Such distinct differences may be mistakenly interpreted as population or species differences. For this reason differences in the lice on the same host species but from different sexes, localities, and subspecies should be examined in any study of this kind.

### Measurements and Standardized Mensuration System

Twelve different characters were measured on each individual, the number being determined on the basis of experience in previous studies. In the study of the *Hoplopleura arboricola* complex (Kim, 1962), 30 characters were measured and analyzed for intercharacter correlation. All the characters used in the present study were shown in earlier studies to be useful in discrimination and to have the least intercharacter correlations.

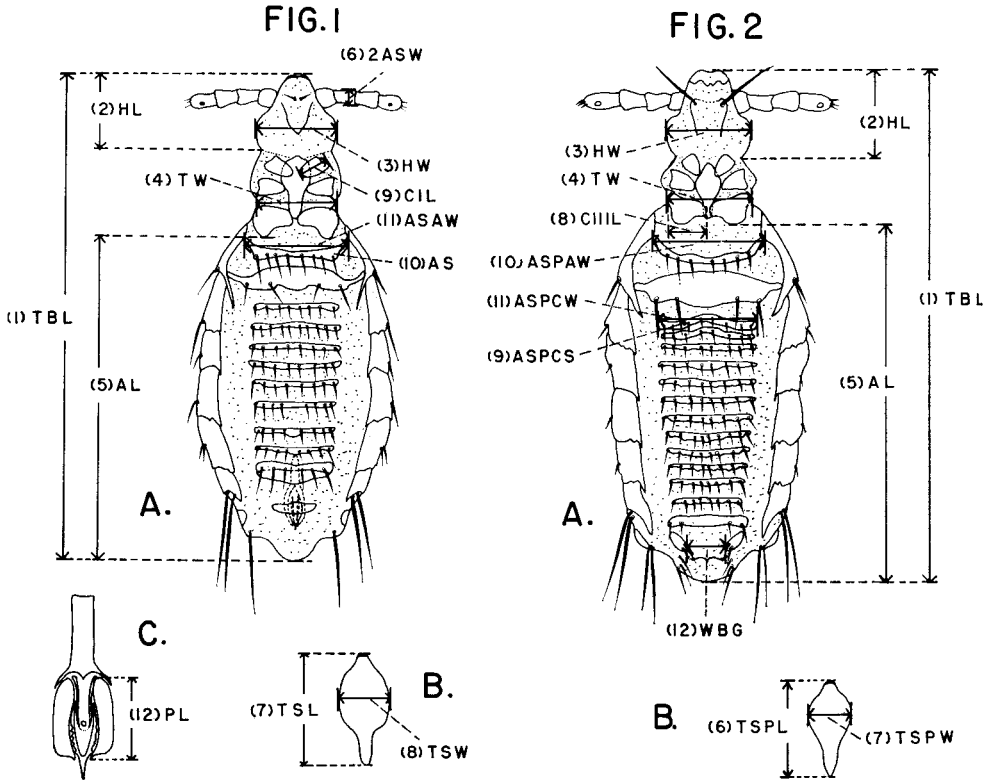
TABLE 1. RELIABILITY OF MEASUREMENTS FOR 12 CHARACTERS MEASURED OF MALE *Hoplopleura hesperomydis* BY K. C. KIM. C.V.% = COEFFICIENT OF VARIATION IN PER CENT.

Characters	Specimen 1				Specimen 2							
	(1)*	(2)*	(3)*	X	S	C.V.%	(1)*	(2)*	(3)*	X	S	C.V.%
Total body length	** 1,920.00	1,908.00	1,908.00	1,912.00	6.928	0.36	** 1,956.00	1,956.00	1,932.00	1,948.00	13.856	0.71
Head length	135.00	140.98	143.64	139.87	4.425	3.16	132.00	140.98	138.32	137.10	4.613	3.36
Head width	122.50	133.00	130.34	128.61	5.458	4.24	120.00	127.68	127.68	125.12	4.434	3.54
Thorax width	172.50	183.00	180.88	178.79	5.552	3.10	155.00	170.24	167.58	164.27	8.141	4.96
Abdomen length	1,332.00	1,308.00	1,284.00	1,308.00	24.000	1.83	1,296.00	1,356.00	1,296.00	1,316.00	34.641	2.63
2nd anten. seg. width	23.40	22.61	22.61	22.87	0.456	1.99	18.20	18.62	18.62	18.48	0.243	1.31
Thoracic stern. pl. length	93.60	95.76	95.76	95.04	1.247	1.31	109.20	113.05	110.09	110.78	2.016	1.81
Thoracic stern. pl. width	66.30	66.50	67.83	66.88	0.832	1.24	59.80	59.80	62.51	60.70	1.565	2.58
Coxa I length	52.00	50.54	50.54	51.03	0.843	1.65	54.60	53.20	53.20	53.67	0.808	1.51
Abd. stern. pl. A (setae)	8	8	8	8.00	0.000	0.00	8	8	8	8.00	0.000	0.00
Abd. stern. pl. A width	160.00	156.94	163.20	160.05	3.130	1.96	175.00	156.94	156.94	162.96	10.427	6.40
Paramere length	55.90	42.56	45.22	47.89	7.060	14.74	71.50	49.21	43.89	54.87	14.648	26.70

\* (1) March 3, 1962, (2) March 20, 1962, (3) January 7, 1963.

\*\* All units are in microns.

HOPLOPLEURA HESPEROMYDIS (OSB.)



FIGS. 1, 2. Standardized Mensuration System of *Hoplopleura hesperomydis* complex. Fig. 1, male. A. Ventral side of body. B. Thoracic sternal plate. C. Male genitalia. Fig. 2, female. A. Ventral side of body. B. Thoracic sternal plate.

Characters measured for the males (Figs. 1A, 1B, 1C) were:

Number	Characters	Abbrev.	Objective	Side viewed*
1.	Total body length	TBL	10×	v
2.	Head length	HL	10×	d
3.	Head width	HW	20×	d
4.	Thorax width	TW	20×	d
5.	Abdomen length	AL	20×	d
6.	Second antennal segment width	2ASW	43×	v
7.	Thoracic sternal plate length	TSL	43×	v
8.	Thoracic sternal plate width	TSW	43×	v
9.	Coxa I length	CIL	43×	v
10.	Abdominal sternal plate A (No. of setae)	AS	20×	v
11.	Abdominal sternal plate A width	ASAW	20×	v
12.	Paramere length	PL	43×	v

\* d—dorsal, v—ventral.

Characters measured for the females (Figs. 2A, 2B) were:

Number	Characters	Abbrev.	Objective	Side viewed*
1.	Total body length	TBL	10×	v
2.	Head length	HL	10×	d
3.	Head width	HW	20×	d
4.	Thorax width	TW	20×	d
5.	Abdomen length	AL	20×	d
6.	Thoracic sternal plate length	TSPL	43×	v
7.	Thoracic sternal plate width	TSPW	43×	v
8.	Coxa III length	CIIL	43×	v
9.	Abdominal sternal plate C (No. of setae)	ASPCS	20×	v
10.	Abdominal sternal plate A width	ASPAW	43×	v
11.	Abdominal sternal plate C width	ASPCW	43×	v
12.	Width between gonopods	WBG	43×	v

\* d—dorsal, v—ventral.

Before the measurements were taken, all the slides of the louse samples were mixed without identification of the louse species. After the measurements were recorded, the slides were reidentified, and the data were regrouped according to the louse species. In this way biases due to changes in measurement technique and progressive and personal errors can be minimized.

All measurements were made by Ke Chung Kim during a period of several days. One character was measured on all individuals, then a second character was measured, and so on through the list of 12 characters.

All measurements were made by means of an American Optical phase-contrast microscope equipped with a linear-graduated ocular micrometer that had been calibrated from a standard stage micrometer to the nearest 0.1 micron. A mechanical stage attached to the microscope was used to increase the speed and precision of taking measurements.

Before measuring a character on each specimen, the correct magnification for that character was set and checked. The specimen was focused on the correct side and the character was always in focus. In this study the micron was the unit of measurement in the analysis; however, use of direct micrometer units throughout the analysis is recommended. These units may be converted to microns after the analysis.

Errors and biases in measurement may arise from personal experience, personal visual aberrations, measuring habits, and inadequacies of the mensuration system. The reliability of measurements should be tested and the results presented in any study of this kind.

The reliability of measurements in this study was assessed for each of 12 characters. Two individuals of *H. hesperomydis* were measured on all 12 characters at three widely spaced times. The results are shown in Table 1. PL is the most unreliable, with coefficients of variation of 14.7% and 26.7% in the two samples.

### *Statistical and Computational Techniques*

Most of the computations were done on the Control Data 1604 Computer, but some of the simpler procedures were carried out on desk calculators.

The sample for each species consisted of louse specimens found on several host animals, in some instances belonging to several host species or subspecies. In order to determine whether the lice of the same species taken from different host animals were different, an analysis of variance was computed for each of 12 characters for comparison of within-host and between-host variation for those louse species (test of homogeneity).

The specimens of *H. hesperomydis* in the University of Minnesota Entomology Collection were collected from *Peromyscus leucopus* and five subspecies of *P. maniculatus*. The test of homogeneity was also done on the louse sample from five subspecies of *P. maniculatus*.

For comparing three louse species, the means and standard deviations were computed for each of 12 characters for 12 males and 12 females of each species. The three species of lice were paired for the purpose of comparison as follows: *H. hesperomydis* (H) vs. *H. onychomydis* (O), *H. hesperomydis* (H) vs. *H. ferrisi* (F), *H. onychomydis* (O) vs. *H. ferrisi* (F).

Student t-tests were carried out for each character, comparing the three species pairwise, for each sex. The tests were computed from log transformed data to assure more homogeneous variances.

In general there is a great overlap between the species in certain characters and less overlap in others. In order to see the amount of overlap between species when one character is used alone to identify the species, the proportion overlap, or the probability of misidentification, was calculated for each character in a particular pair of species.

The proportion overlap or misidentification ( $P$ ) is obtained from the estimate of the distance between character means of the two species, divided by the pooled

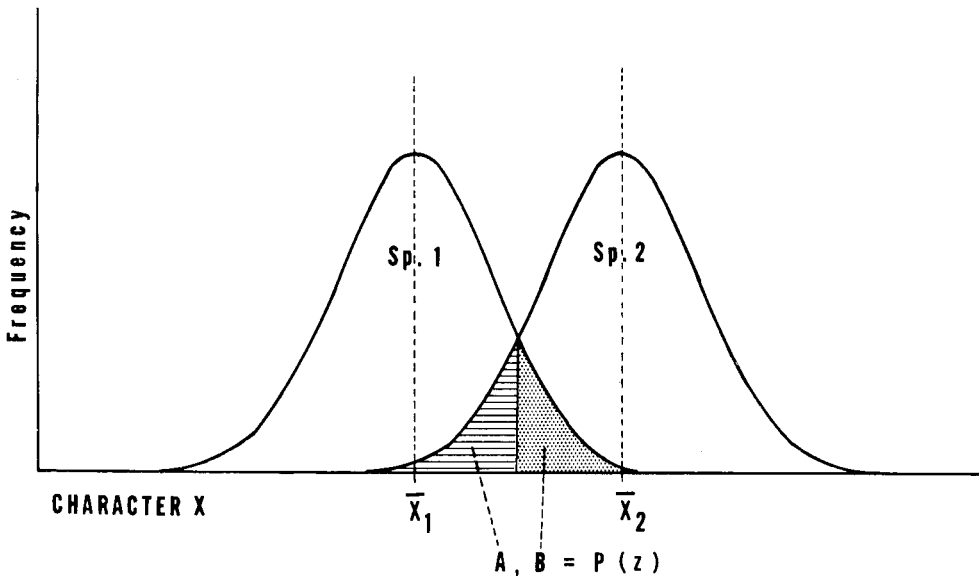


FIG. 3. Diagram showing proportion overlap or probability of misidentification ( $P$ ) using one measured character ( $X$ ) to identify one of two species.

estimate of the common standard deviation for a particular character, i.e.,  $z = (\bar{X}_1 - \bar{X}_2)/2S$ , where  $\bar{X}_1$  and  $\bar{X}_2$  are the character means (log scale) for the two species and  $S$  is the pooled estimate (log scale) of the within-population variation of the character (Fig. 3). The value  $Z$  is then referred to a table of normal probabilities to obtain the per cent overlap ( $P$ ). The per cent is the estimated probability of misidentification if a particular character alone were used to identify lice as one of the two species.

Correlation coefficients for intercharacter correlation were also calculated to find the degree of independence among characters.

Discrimination among the three species of lice was investigated using a combination of characters with low intercharacter correlation and low probability of misidentification. The technique of discriminant analysis was also employed, using all 12 characters simultaneously to discriminate between species. Six discriminant analyses were computed for both males and females.

Most programs were written in Fortran 60 language by Mrs. Mary Kim Bilek. Some

of the programs used are in the library of the University of Minnesota Numerical Analysis Center. All the data including computer outputs and additional information in the programs can be obtained from the senior author, Ke Chung Kim, Department of Entomology, Fisheries, and Wildlife, University of Minnesota, St. Paul, Minnesota 55101.

### Results

*Test of homogeneity for samples of the three louse species.*—Previous studies clearly indicate that there are large differences between populations of lice from different host animals of the same species, different localities and sexes, and that specimens used in any study of this kind should be obtained from a number of host animals rather than a single one (Kim, Brown, and Cook, 1963).

Unless the test of homogeneity is carried out for samples under study, there is no way to assure that the results obtained from samples of a single host animal or of a single locality or of unknown data represent a true picture of the population or species discrimination, rather than individual host

TABLE 2. ANALYSES OF VARIANCE FOR 12 CHARACTERS IN MALES OF THE THREE LOUSE SPECIES OF THE *Hoplopleura hesperomydis* COMPLEX.

Char- acters	<i>H. hesperomydis</i>			<i>H. onychomydis</i>			<i>H. ferrisi</i>		
	MS between	MS within	P	MS between	MS within	P	MS between	MS within	P
TBL	108.00	4,581.60	0.825	2,885.23	3,513.60	0.625	9,684.00	6,826.50	0.375
HL	0.52	44.27	0.900	72.66	27.17	0.175	1.39	26.75	0.375
HW	0.52	24.27	0.625	90.15	75.28	0.375	21.36	10.45	0.175
TW	6.19	46.20	0.375	80.70	206.18	0.825	206.59	101.95	0.175
AL	300.00	652.08	0.825	1,821.47	3,320.64	0.625	1,056.00	6,408.00	0.925
2ASW	3.52	6.90	0.375	3.34	1.13	0.175	4.78	5.01	0.375
TSL	858.52	773.40	0.375	47.18	19.38	0.175	2.34	26.93	0.965
TSW	23.80	14.34	0.175	11.94	7.35	0.375	19.38	8.96	0.175
CIL	40.70	4.54	0.018	3.70	7.67	0.825	13.80	7.55	0.175
AS	0.09	0.08	0.375	0.88	0.10	0.018	0.05	0.09	0.625
ASAW	150.52	214.27	0.375	151.03	110.38	0.375	804.51	513.08	0.375
PL	394.46	39.98	0.018	21.69	7.72	0.175	43.19	32.53	0.375

or locality differences. For this reason it is advisable to investigate the homogeneity of samples under study before conducting any further analysis and drawing any conclusion.

The materials used in this test are as follows: *Hoplopleura hesperomydis*—6 males and 6 females from 2 individual skins of *Peromyscus leucopus*, 6 males and 6 females from 2 individual skins of *P. maniculatus bairdi*; *Hoplopleura onychomydis*—5 males and 5 females from 2 individual skins, 2 males and 3 females from a third skin, and 4 males and 3 females from 7 additional host skins of *Onychomys torridus*; *Hoplopleura ferrisi*—4 males and 4 females from 2 individual skins of *Peromyscus boylii*, 4 males and 4 females from 2 individual skins of *P. eremicus*, 2 and 2 male, 2 and 2 female specimens from 4 host skins of *P. nasutus*, respectively.

A one-way analysis of variance was computed for each of 12 characters of both males and females of three louse species for the purpose of determining whether or not there are any important and consistent differences between lice of the same species found on one individual host animal versus those found on another host animal of same species, or the lice of the same species found on one host species versus those found on another host species, or the lice found on one host subspecies versus those found on another host subspecies of the same

species, or even the lice found in one locality versus those in another locality. The results are given in Tables 2 and 3. A multivariate test for the difference of the 12-dimensional mean vector might have been appropriate but was not attempted in this study.

As shown in Tables 2 and 3, there is no evidence of intraspecific heterogeneity for any of the characters common to both males and females (TBL, HL, HW, TW, and AL). Furthermore, there is also reassuring intraspecific homogeneity for the remaining characters for each sex. Exceptions are CIL and PL for male *hesperomydis*, AS for male *onychomydis*, ASPCS and ASPAW for female *hesperomydis*, and TSPL, ASPAW, and WBG for female *ferrisi*. At this time this rather weak evidence for the existence of subspecies has not been further investigated, and the data for each species have been considered as homogeneous and representative of the respective species.

*Differences among the louse populations on five subspecies of Peromyscus maniculatus.*—In this side-study, the louse populations of *Hoplopleura hesperomydis* on five subspecies of *Peromyscus maniculatus* were investigated to see the difference among them and also to compare these with the differences between the louse populations on *P. leucopus* and *P. maniculatus*.

The subspecies are: *Peromyscus mani-*

TABLE 3. ANALYSES OF VARIANCE FOR 12 CHARACTERS IN FEMALES OF THE THREE LOUSE SPECIES OF THE *Hoplopleura hesperomydis* complex.

Char- acters	<i>H. hesperomydis</i>			<i>H. onychomydis</i>			<i>H. ferrisi</i>		
	MS between	MS within	P	MS between	MS within	P	MS between	MS within	P
TBL	8,112.00	21,292.80	0.625	9,571.63	55,696.00	0.965	26,084.00	40,504.00	0.625
HL	0.52	28.02	0.825	31.62	45.59	0.625	50.87	90.03	0.625
HW	42.19	27.60	0.175	33.44	40.09	0.625	8.68	24.02	0.825
TW	25.52	60.10	0.625	83.08	22.01	0.175	6.25	88.28	0.965
AL	9,408.00	15,729.60	0.375	11,155.63	51,280.00	0.965	29,840.00	42,912.00	0.625
TSPL	3.52	21.10	0.625	32.38	36.20	0.625	39.67	8.08	0.038
TSPW	20.28	14.20	0.375	10.94	0.33	0.003	5.77	13.09	0.625
CIIL	3.52	63.35	0.825	29.29	9.04	0.175	8.68	10.03	0.375
ASPCS	1.33	0.17	0.018	0.36	0.57	0.625	0.91	1.03	0.375
ASPAW	1,065.97	124.07	0.018	87.64	153.30	0.625	262.75	48.85	0.038
ASPCW	240.30	73.36	0.175	30.01	69.77	0.825	433.47	467.84	0.375
WBG	1.27	14.56	0.825	8.74	7.62	0.375	31.78	6.44	0.038

*culatus bairdi* (from Rosemount, Minnesota, 1955), *P. m. gracilis* (Basswood Lake, Minnesota, 1953), *P. m. osgoodi* (Douglas, Wyoming, 1955), *P. m. oreas* (Kid Valley, Washington, 1956), and *P. m. rubidus* (Beverly Beach, Oregon, 1956).

Two male and two female specimens from each host subspecies were used for the analysis. Each louse specimen represented an individual host. The measurements of 12 characters for 10 males and 10 females are given in Tables 4 and 5.

An analysis of variance was computed for each of 12 characters for 10 specimens of *H. hesperomydis* from five host subspecies for both sexes, in order to see the

differences among the louse populations caused by differences in host subspecies and localities. The results of the analyses of variance for 12 characters of both male and female specimens are given in Table 6.

There is no indication of heterogeneity among females of *H. hesperomydis* associated with subspecies of the host, even for the ASPCS and ASPCW that seem to indicate some heterogeneity in Table 5. On the other hand, there is some evidence of heterogeneity among the males in the characters, HW, TW, 2ASW, and CIL (Table 4). The cause of such heterogeneity among males, while females showed no heterogeneity, was not explored in this study.

TABLE 4. MEASUREMENTS OF CHARACTERS OF MALES OF *Hoplopleura hesperomydis* FROM FIVE SUBSPECIES OF *Peromyscus maniculatus*.\*

Characters	<i>P. m. bairdi</i>		<i>P. m. gracilis</i>		<i>P. m. osgoodi</i>		<i>P. m. oreas</i>		<i>P. m. rubidus</i>	
	1	2	3	4	5	6	7	8	9	10
TBL	1,776.00	1,860.00	1,824.00	1,620.00	1,812.00	1,944.00	1,680.00	1,824.00	1,644.00	1,680.00
HL	135.66	140.98	148.96	133.00	143.64	140.98	148.96	146.30	138.32	138.32
HW	114.38	122.36	133.00	133.00	135.66	138.32	127.68	133.00	117.04	122.36
TW	143.64	138.32	143.64	140.98	162.26	159.60	154.28	151.62	138.32	140.98
AL	1,248.00	1,284.00	1,224.00	1,068.00	1,188.00	1,272.00	1,104.00	1,236.00	960.00	1,092.00
2ASW	25.27	25.27	22.61	19.95	23.94	21.28	19.95	21.28	22.61	21.28
TSL	89.11	101.08	106.40	101.08	102.41	106.40	93.10	97.09	99.75	98.42
TSW	53.20	53.20	55.86	55.86	57.19	55.86	50.54	50.54	50.54	53.20
CIIL	47.88	50.54	39.90	37.24	45.22	45.22	42.56	46.55	42.56	42.56
AS	8	8	8	8	8	8	7	8	8	8
ASAW	156.94	167.58	159.60	167.58	154.28	159.60	162.26	154.28	146.30	156.94
PL	70.49	83.79	78.47	71.82	73.15	75.81	70.49	70.49	74.48	71.82

\* All measurements are in microns.



TABLE 5. MEASUREMENTS OF CHARACTERS OF FEMALES OF *Hoplopleura hesperomydis* FROM FIVE SUBSPECIES OF *Peromyscus maniculatus*.\*

Characters	<i>P. m. bairdi</i>		<i>P. m. gracilis</i>		<i>P. m. osgoodi</i>		<i>P. m. oreas</i>		<i>P. m. rubidus</i>	
	1	2	3	4	5	6	7	8	9	10
TBL	2,292.00	2,364.00	2,232.00	2,256.00	2,196.00	2,352.00	2,232.00	2,124.00	2,232.00	2,088.00
HL	146.30	140.98	135.66	146.30	133.00	146.30	143.64	133.00	138.32	135.66
HW	122.36	117.04	135.66	140.98	127.68	140.98	133.00	130.34	119.70	127.68
TW	146.30	148.96	154.28	159.60	154.28	162.26	159.60	140.98	143.64	138.32
AL	1,716.00	1,752.00	1,632.00	1,644.00	1,560.00	1,716.00	1,620.00	1,548.00	1,608.00	1,536.00
TSPL	97.09	103.74	98.42	109.06	105.07	103.74	105.07	98.42	103.74	98.42
TSPW	54.53	53.20	55.86	57.19	55.86	57.19	53.20	50.54	55.86	50.54
CHIL	67.83	85.12	67.83	71.82	77.14	74.48	73.15	69.16	77.14	70.49
ASPCS	8	8	8	7	7	7	8	8	8	7
ASPAW	148.96	159.60	155.61	159.60	159.60	159.60	159.60	152.95	159.66	162.26
ASPCW	146.36	162.26	172.90	164.92	159.94	172.90	159.60	155.61	172.90	155.61
WBC	70.49	69.16	79.80	74.48	75.81	77.14	79.80	75.81	75.81	75.81

\* All measurements are in microns.

*Comparison of the three louse species on individual characters.*—The mean and standard deviation were computed for 12 characters in both males and females of the three species in order to compare the three louse species, as shown in Table 7 for the male and Table 8 for the female.

Tests of significance were made for each of 12 characters comparing each sex of the three louse species pairwise. The student t-tests are given in Table 9. There is little doubt of the difference among *H. onychomydis* and the other species, and that the difference between *H. hesperomydis* and *H. ferrisi* is not as marked, but is nevertheless well established, as shown in the table.

The proportion of overlap is shown in Table 10 as the probability of misidentification (*P*). It would be close to the maximum of 50% for distinguishing between *H. hesperomydis* and *H. ferrisi* for one-third of 12 characters, for both male and female lice. On the other hand, the correct identification of the lice to one of the two species, between *H. hesperomydis* and *H. onychomydis* for males, and between *H. onychomydis* and *H. ferrisi* for females, is much more certain, and the discriminations between *H. onychomydis* and *H. ferrisi* for both sexes, especially for males, and between *H. hesperomydis* and *H. onychomydis* for females, are even better.

TABLE 6. ANALYSIS OF VARIANCE OF CHARACTERS OF LOUSE POPULATIONS OF *Hoplopleura hesperomydis* FROM FIVE SUBSPECIES OF *Peromyscus maniculatus*.

Characters	Males			Characters	Females		
	MS between	MS within	<i>P</i>		MS between	MS within	<i>P</i>
TBL	14,100.00	8,813.00	0.33	TBL	9,554.00	6,250.00	0.33
HL	29.36	29.72	0.43	HL	13.09	43.87	0.86
HW	136.20	12.74	0.02	HW	117.80	30.43	0.09
TW	170.50	5.66	0.00	TW	100.10	47.41	0.23
AL	17,050.00	6,754.00	0.19	AL	8,186.00	3,614.00	0.22
2ASW	6.46	1.77	0.10	TSPL	5.93	23.17	0.89
TSL	40.51	20.52	0.24	TSPW	8.58	4.07	0.23
TSW	13.09	0.89	0.01	CHIL	16.63	38.21	0.78
CIL	30.25	3.01	0.02	ASPCS	0.35	0.20	0.29
AS	0.10	0.10	0.49	ASPAW	13.89	18.04	0.60
ASAW	44.93	38.21	0.45	ASPCW	70.14	88.80	0.60
PL	12.21	23.53	0.72	WBC	20.61	4.78	0.08

TABLE 7. MEANS AND STANDARD DEVIATIONS OF THREE SPECIES OF THE *Hoplopleura hesperomydis* COMPLEX (MALES).\*

Characters	<i>Hoplopleura hesperomydis</i> ♂		<i>Hoplopleura onychomydis</i> ♂		<i>Hoplopleura ferrisi</i> ♂	
	$\bar{X}$	S	$\bar{X}$	S	$\bar{X}$	S
TBL	1,781.00	64.614	1,707.27	56.563	1,878.00	87.211
HL	128.96	6.348	141.95	7.065	128.75	5.055
HW	113.54	4.702	122.60	9.095	117.04	3.665
TW	135.21	6.524	137.11	11.977	147.92	11.423
AL	1,201.00	77.170	1,111.64	50.706	1,284.00	70.345
2ASW	20.91	2.568	23.22	1.496	20.48	2.226
TSL	105.31	27.949	106.04	5.770	101.18	4.498
TSW	53.19	3.898	62.03	3.106	58.39	3.438
CIL	48.21	2.796	47.52	2.386	51.03	3.042
AS	7.92	0.289	7.91	0.701	7.91	0.289
ASAW	157.29	14.439	198.29	11.433	171.04	24.343
PL	68.78	8.497	67.71	3.835	70.63	5.953

\* All measurements are in microns.

Table 10 shows that the characters of greatest use generally in distinguishing the three species are as follows:

For male—TBL, HL, HW, AL, TSW, ASAW;

For female—HL, HW, TW, AL, TSPL, CIIL, WBG.

It is clear that the use of a single character for distinguishing the louse species simultaneously is not adequate in most instances (Kim, Brown, and Cook, 1963). The simultaneous use of two or more characters in combination for identification of

the species will be considered in a later section.

*Selection of taxonomic characters.*—Correlation coefficients among the characters were computed for each of the 12 characters of three species to find the degree of independence among characters. A correlation matrix for each sex of each species was prepared (log scales).

From the correlation matrices, groups of characters with sample correlation uniformly less than 0.30 were chosen:

For males,

*H. hesperomydis*—TBL, HL, HW,

TABLE 8. MEANS AND STANDARD DEVIATIONS OF THREE SPECIES OF THE *Hoplopleura hesperomydis* COMPLEX (FEMALES).\*

Characters	<i>Hoplopleura hesperomydis</i> ♀		<i>Hoplopleura onychomydis</i> ♀		<i>Hoplopleura ferrisi</i> ♀	
	$\bar{X}$	S	$\bar{X}$	S	$\bar{X}$	S
TBL	2,346.00	141.755	2,107.64	192.993	2,290.00	191.237
HL	132.29	5.052	147.99	6.325	137.08	8.908
HW	114.79	5.379	127.92	6.119	118.54	4.454
TW	145.63	7.547	157.42	6.815	157.50	8.118
AL	1,766.00	123.105	1,480.36	187.697	1,702.00	198.361
TSPL	101.94	4.416	111.23	5.888	107.47	4.086
TSPW	53.30	3.841	63.51	2.139	58.18	3.332
CIIL	72.26	7.610	82.34	4.140	76.16	3.109
ASPCS	7.50	0.522	7.91	0.701	7.50	1.000
ASPAW	157.84	14.481	169.27	11.271	167.92	10.353
ASPCW	158.53	9.409	154.76	7.340	160.64	21.378
WBG	63.81	3.654	106.16	2.841	66.84	3.654

\* All measurements are in microns.

TABLE 9. STUDENT T-TEST FOR EACH OF 12 CHARACTERS OF THE THREE SPECIES OF THE *Hoplopleura hesperomydis* COMPLEX.\* FIGURES ARE PROBABILITY VALUES FOR THE STUDENT T-TEST FOR SIGNIFICANCE OF MEANS.

Characters	Males			Characters	Females		
	H vs. O	H vs. F	O vs. F		H vs. O	H vs. F	O vs. F
TBL	0.009	0.006	0.000	TBL	0.003	0.430	0.030
HL	0.000	0.500	0.000	HL	0.000	0.130	0.004
HW	0.007	0.050	0.080	HW	0.000	0.075	0.000
TW	0.500	0.003	0.035	TW	0.000	0.001	0.000
AL	0.004	0.013	0.000	AL	0.000	0.330	0.015
2ASW	0.020	0.500	0.003	TSPL	0.000	0.004	0.090
TSL	0.500	0.500	0.040	TSPW	0.000	0.003	0.000
TSW	0.000	0.002	0.013	CIIL	0.001	0.100	0.000
CIL	0.500	0.035	0.008	ASPCS	0.135	0.500	0.240
AS	0.500	0.500	0.500	ASPAW	0.043	0.050	0.500
ASAW	0.000	0.040	0.001	ASPCW	0.330	0.500	0.500
PL	0.500	0.500	0.200	WBG	0.000	0.050	0.000

\* The tests were computed on log transformed data.

TW, TSW, TSL, CIL;

*H. onychomydis*—TBL, HW, TW, TSW, CIL;

*H. ferrisi*—TBL, HW, TW, 2ASW, TSL.

For females,

*H. hesperomydis*—TBL, HL, HW, TSPL, TSPW, ASPCS;

*H. onychomydis*—TBL, HW, TW, TSPL, TSPW, CIIL;

*H. ferrisi*—HW, TW, TSPL, CIIL, or TBL, CIIL, ASPCS.

Examination of the correlation matrices shows that TBL is consistently highly correlated with AL throughout the species for both sexes as one may predict, and also TBL is highly correlated with HL in most cases. In females of *H. onychomydis* HL is highly correlated with AL, for which an explanation cannot be given at present. In males TSW is highly correlated with TW rather consistently, and in females TSPL is consistently highly correlated with WBG.

The characters that are mutually independent, as listed above, were individually checked against the characters with low

TABLE 10. PROBABILITIES OF MISIDENTIFICATION ( $P$ ) OF THREE SPECIES OF THE *Hoplopleura hesperomydis* COMPLEX. FIGURES DENOTE ESTIMATED PROPORTION OF LICE MISIDENTIFIED.

Characters	Males			Characters	Females		
	H vs. O	H vs. F	O vs. F		H vs. O	H vs. F	O vs. F
TBL	0.27	0.27	0.13	TBL	0.24	0.43	0.32
HL	0.16	0.50	0.14	HL	0.09	0.37	0.24
HW	0.27	0.34	0.35	HW	0.13	0.35	0.19
TW	0.47	0.25	0.32	TW	0.21	0.22	0.50
AL	0.25	0.29	0.08	AL	0.19	0.42	0.28
2ASW	0.29	0.47	0.24	TSPL	0.18	0.26	0.36
TSL	0.46	0.48	0.32	TSPW	0.06	0.25	0.17
TSW	0.11	0.24	0.29	CIIL	0.21	0.36	0.20
CIL	0.45	0.32	0.27	ASPCS	0.37	0.49	0.40
AS	0.49	0.50	0.49	ASPAW	0.33	0.34	0.48
ASAW	0.06	0.36	0.22	ASPCW	0.42	0.49	0.45
PL	0.48	0.44	0.40	WBG	0.00	0.34	0.00

Species	P		Correlation Coefficients	
	TBL	TSW	TBL:TSW	h: -0.10 o: 0.20 r: 0.45
h vs. o	0.27	0.11		
h vs. f	0.27	0.24		
f vs. o	0.13	0.29		

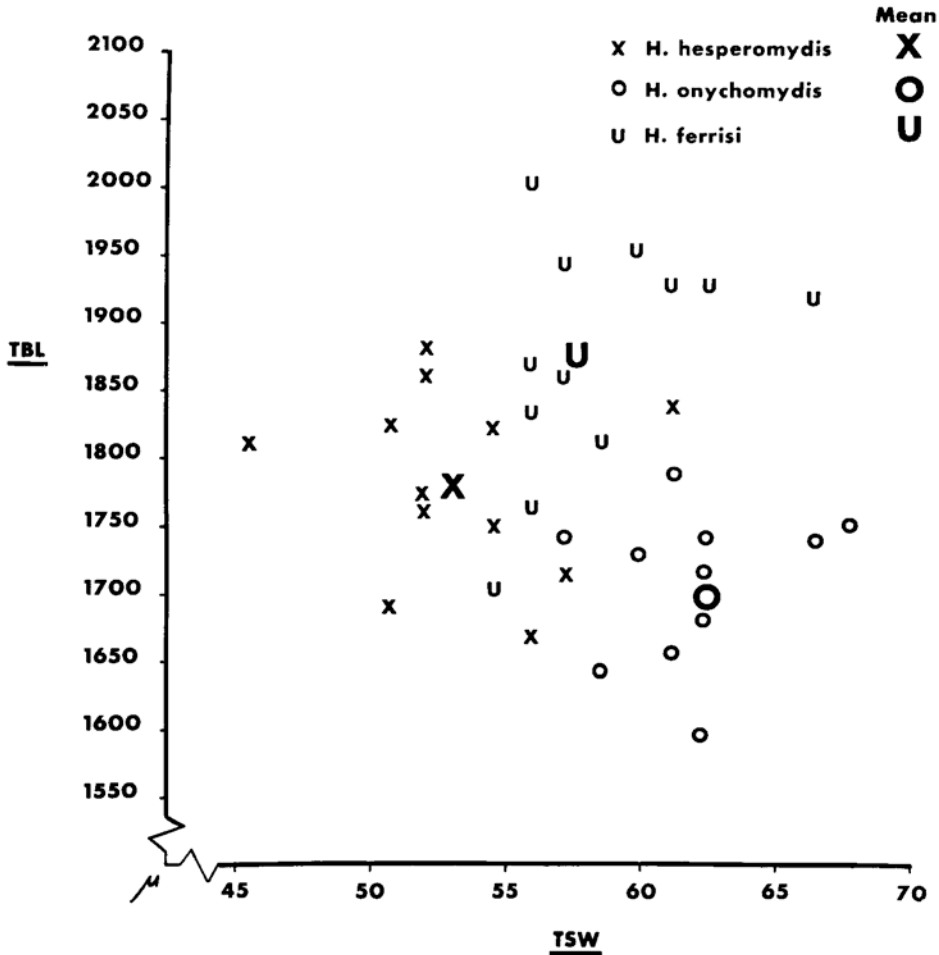


FIG. 4. Scatter diagram showing discrimination among males of three species of *Hoplopleura*.

probability of misidentification in Table 10. The characters that show consistently low probability of misidentification ( $P < 0.30$ ) and are mutually independent of each other are, for males, TBL and TSW; for females TSPL (except for a pair, O vs. F,  $p = 0.36$ ) and TSPW. Those characters can be used as diagnostic taxonomic characters. If the characters listed above do not perform

simultaneous identification of three species, the following characters with generally consistently low probability of misidentification ( $P < 0.30$ ) and intercharacter correlation coefficient (C.C.C.) less than 0.30 will better discriminate between species pairs:

For males,  
H vs. O : TBL, HW, TSW;

H vs. F : TBL, TW, TSW (except for C.C.C. = 0.45 between TBL and TSW for F);

O vs. F : TBL, TSW.

For females,

H vs. O : TBL, HW, TSPW, TSPL;

H vs. F : TW, TSPL, TSPW, WBG (except for C.C.C. = 0.47 between TW and WBG and  $P = 0.41$  for H);

O vs. F : TBL (except for  $P = 0.32$ ), HW, CIIL, WBG.

### Discrimination Among Three Species

Using two characters chosen for each sex, based on  $P$  values and correlations presented earlier, the three species are plotted in Figures 4 and 5. It can be observed that they are more or less distinct and recognizable in both sexes, although there is not enough separation to positively identify each individual without error. *H. onychomydis* is quite distinct from either *H. ferrisi* or *H. hesperomydis*, as already clearly demonstrated by a set of qualitative characters (Cook and Beer, 1959).

A complete discrimination between *H. ferrisi* and *H. hesperomydis*, using these two characters, cannot be obtained. However, the discrimination between *H. ferrisi* and *H. hesperomydis* or *H. ferrisi* and *H. onychomydis* can be improved by using another set of characters suited for a particular pair of species. By using TBL and TW (H vs. F:  $P$  value; TBL = 0.27, TW = 0.25; correlation between TBL and TW: H = 0.03, F = -0.13), the discrimination of males between *H. ferrisi* and *H. hesperomydis* can be refined as shown in Figure 6. Likewise, the discrimination of females between *H. ferrisi* and *H. hesperomydis* or between *H. ferrisi* and *H. onychomydis* can be improved by TW and WBG (H vs. F:  $P$  value; TW = 0.22, WBG = 0.34; correlation between TW and WBG: F = 0.02, H = 0.47) and CIIL and WBG (O vs. F:  $P$  value; CIIL = 0.20, WBG = 0.00; correlation between CIIL and WBG: O = 0.00, H = 0.30), respectively.

The data for the discrimination of fe-

males between *H. onychomydis* and *H. ferrisi* are plotted in Figure 7. There is a complete discrimination between *H. onychomydis* and *H. ferrisi* with CIIL and WBG.

The technique of discriminant analysis was also employed, using all 12 characters simultaneously, to discriminate between the species (considering the two sexes separately), and the results of the less formal analysis described above were confirmed. Tests of significance (using the Hotelling  $T^2$  statistic) showed that both male and female of *H. onychomydis* differ significantly from the other two species at the 0.0005 significance level. However, the tests of significance for comparing *H. hesperomydis* with *H. ferrisi* yielded probability values of 0.07 for males and 0.25 for females.

Discriminant functions were computed for each of the six discriminations (between the species, pairwise, for the three species, for males and for females), and the values of the discriminant functions were computed for each of the individuals in each group. There was complete separation of individuals for each of the six analyses, but the degree of separation in the cases of *H. hesperomydis* and *H. ferrisi* was rather small. Cross validation on new samples would almost certainly show a substantial number of individuals misidentified by the discriminators for *H. hesperomydis* versus *H. ferrisi*, but very few if any would be misidentified in the case of *H. onychomydis* versus the other two species.

### A Posteriori Taxonomic Characters

*Recognition of species.*—The recognition and identification of species is one of the basic tasks of the systematist. Furthermore, he must provide such species with appropriate names and a description or taxonomic characters by which other workers will subsequently be able to recognize them. However, species are frequently not easy to define and to recognize.

Species are dynamic biological units (also the taxonomic units) which exist in nature,

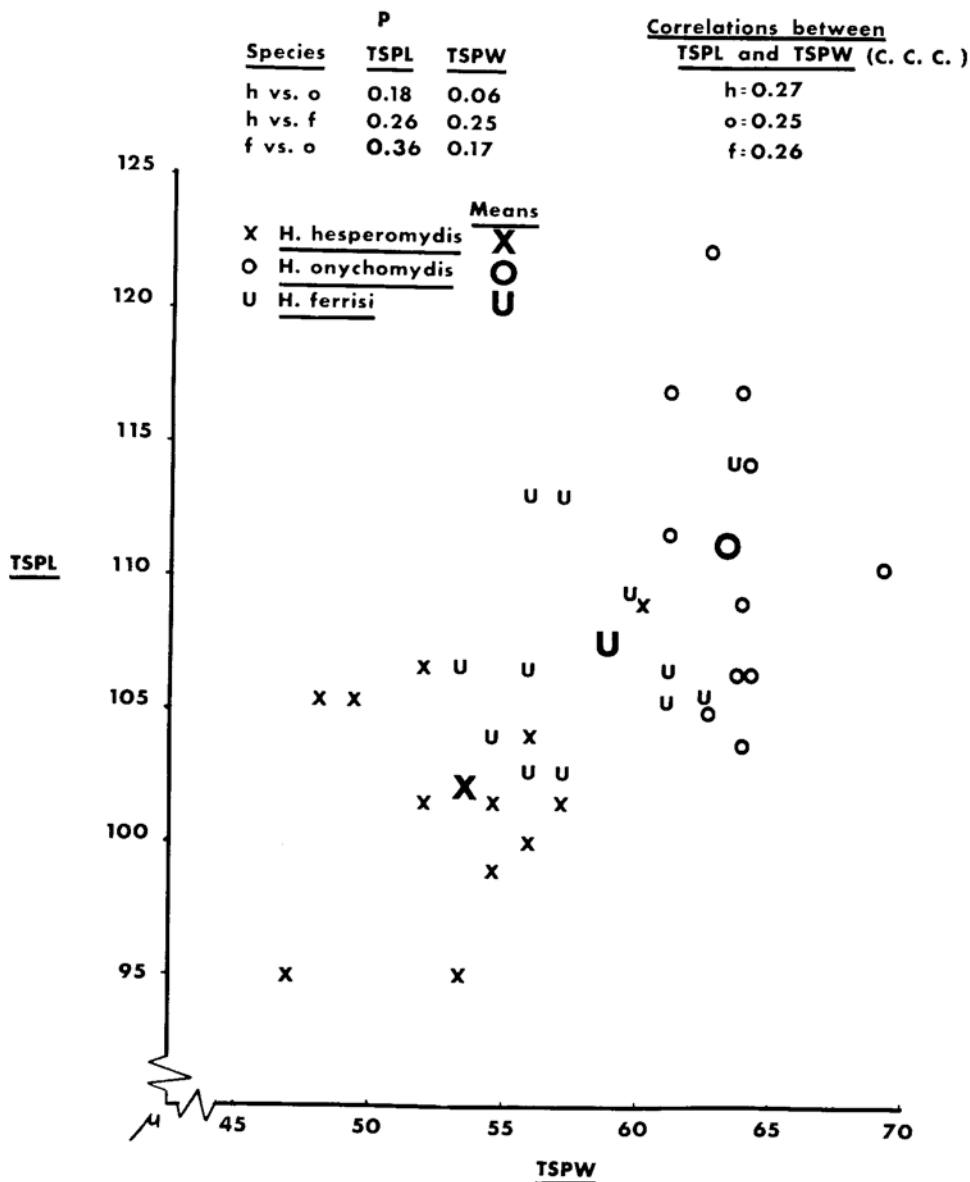


FIG. 5. Scatter diagram showing discrimination among females of three species of *Hoplopleura*.

regardless of our capability to recognize them, and are the products of a long evolutionary process of selection and reproductive isolation involving in bisexual organisms a complex system of intrinsic and extrinsic factors. A species character is generally defined as "any attribute of a species that differentiates it from other species,

and that is reasonably constant, so that a species can be recognized by it" (Mayr, 1963). A species character is a by-product of the genetic and biological discontinuity resulting from selection and reproductive isolation. Among species characters, morphological characters are most convenient and useful for diagnostic purposes, partic-

ularly in preserved specimens. For this reason systematists have usually relied on morphological characters in defining and recognizing species.

The publication in 1937 of the first edition of Dobzhansky's *Genetics and the Origin of Species* marked the advent of the modern study of species and speciation. Prior to this, species had been recognized more or less solely through gross morphological differences. Even at present systematists recognize undiscovered species by thorough analysis of the microanatomy of the organisms, such as insect genitalia. For instance, Sabrosky (1949) found 12 new species in the *Leptocera lutosa* (Fall.) complex, the species easily distinguishable on the basis of differences in the genitalia of both sexes.

In some species the morphological characters of immature or egg stages are more useful in recognizing species than those of the adult stages, e.g., eggs of the *Anopheles maculipennis* complex (Diptera), or immature stages of the *Hoplopleura hesperomydis* complex (Anoplura).

When a systematist recognizes species by morphological differences in his materials, his species recognition is founded on the assumption that the specimens examined are representative of the species at a given time. A certain fraction of the genetic differences between populations may be reflected in morphological traits, and hence, the morphological description reflects reasonably accurately the magnitude of the genetic differences between species (Dobzhansky, 1937). However, it is generally accepted by biologists that the genetic changes and discontinuity resulting from reproductive isolation will yield detectable changes in biochemical, physiological, cytological, ecological, behavioral, morphological, or other traits, but such changes will not necessarily be accompanied by detectable morphological changes. Thus, morphological characters may not be primary criteria for distinguishing species, but may play a secondary role as a taxonomic tool in the recognition of species. Furthermore, studies

of very diversified species or so-called species complexes by field biologists, ecologists, geneticists, physiologists, and others have revealed that cryptic or sibling species are much more common than one may suppose. Cryptic species are defined as species morphologically so similar, if not identical, that their identity is uncertain and unrecognized unless confirmed by other evidence (Walker, 1964). Poor response to the recognition of this phenomenon by systematists may have been an important source of taxonomic errors and the center of criticism by other biological disciplines.

Cryptic species are discovered in various ways. Examples of different biological attributes that may aid in distinguishing and discovering cryptic species are well summarized in Dobzhansky (1937), Brown (1959), and Mayr (1963).

Cryptic species have usually been shown to have some differences in morphological characters, minor or major, when the morphology of the organisms is thoroughly studied, once the cryptic species are recognized. However, it is important to note that some cryptic species cannot be identified from preserved specimens, and some may never be (Walker, 1964). In many instances, distinguishing between cryptic species is of economic significance. An example is the *Anopheles maculipennis* complex, where correct identification of species has great economic and public health significance in malaria eradication. When cryptic species are recognized, but cannot be distinguished from other species by visible morphological characters, systematists must provide convenient and useful diagnostic tools which will facilitate subsequent recognition of such taxa.

Cryptic species may be revealed through statistical analysis of groups of specimens from various sources (e.g., host species or localities). The distinctness of imputed cryptic species can be substantiated in some cases by quantitative or statistical studies, even if qualitative morphological differences between species are small, if not ab-

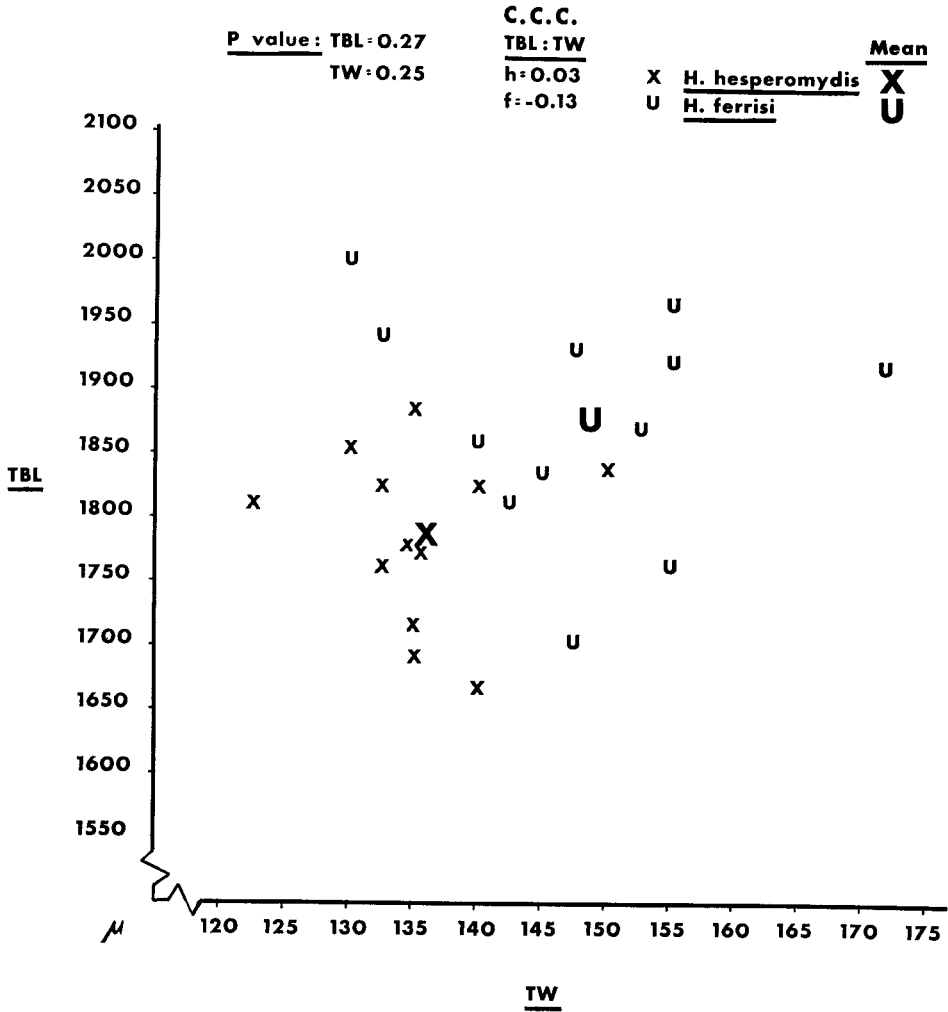


FIG. 6. Scatter diagram showing discrimination between males of two species of *Hoplopleura*.

sent. Some well-known examples of such studies are discussed below.

In the genus *Drosophila* most of the species complexes contain many cryptic species. The best example may be found in the most intensively studied species-group, *D. pseudoobscura* Frolora and *D. persimilis* Dobzhansky and Epling. Lancefield (1929) originally discovered differences between two stocks of *D. pseudoobscura* (= *D. obscura* of European authors), in the shape and size of the Y-chromosome and in genetic behavior (breeding experiments). Since this discovery a number of differences

in various biological attributes have been found by other workers. A quantitative study of the morphological differences between the "races" of *D. pseudoobscura* by Mather and Dobzhansky (1939) typifies the statistical work of this kind at the specific level. Mather and Dobzhansky used the analysis of variance for the purpose of investigating variation among individuals of the same strain and among strains of the same race. They also used the discriminant function of Fisher (1936) to study racial differences. Races A and B were found to be distinct entities through this study. These



two races were eventually raised to the rank of full species, when they were found to coexist over a wide area without interbreeding (Dobzhansky and Epling, 1944). Reed, Williams, and Chadwick (1942) and Reed and Reed (1948) also confirmed the anatomical distinctness of the two species by using a special character index from several wing measurements. Later Rizki (1951) and Spassky (1957) showed distinct differences between the species in the shape of the male genitalia and in a group of other minor morphological characters.

Lorkovic (1942) made a simple quantitative study of the blues, *Everes* (Lepidoptera: Lycaenidae), and has recognized three physiological and morphological forms as three distinct species, *Everes argiades* Pallas, *E. alcetas* Hoffmannsegg, and *E. decolorata* Staeger, by using a group of characters, i.e., wing width, anal spot of wing, and other characters in the wing.

The distinctness of two subspecies of darters, *Boleosoma nigrum nigrum* (Storer) and *B. nigrum olmstedii* (Rafinesque) (Pisces), was confirmed by a quantitative study, using Fisher's discriminant function (Stone, 1947). The specimen indices (= linear discriminant functions) based on four chosen characters were shown in two histograms. Stone suggested that since these two subspecies appeared to be so distinct, *B. nigrum nigrum* and *B. nigrum olmstedii* should be elevated to specific status.

An application of the discriminant function to taxonomy was illustrated in a study of the *Culicoides varipennis* complex by McGuire and Wirth (1958). Discriminant functions were computed for five subspecies of *C. varipennis* (Coq.), using four characters which were selected by the taxonomists as good taxonomic characters, in order to test the validity of those subspecies.

Kim, Brown, and Cook (1963) demonstrated that louse populations of *Enderleinellus suturalis* (Osborn) from *Citellus tridecemlineatus*, *C. franklini*, and *C. harrisi* are distinct taxa. By using a combination of characters selected on the basis of

low probability of misidentification and low intercharacter correlation, these three louse populations were clearly distinguishable. In this study it was suggested that louse populations from other species of ground squirrels, which are known to harbor the *E. suturalis* complex, might be as distinct as these populations, and might be good species or subspecies. Kim (1966), in a more recent paper, stated that differences among nymphs of the *E. suturalis* complex from the three host species are not distinct enough to be useful in distinguishing the three taxa. Accordingly, he suggested that differences among the three taxa of *E. suturalis* complex are more subspecific than specific.

Upon studying various populations of a common flea beetle, *Chaetocnema concinna* Marsh, s. l., from different localities, Lubischew (1962) discovered two new species which had been confused with *Ch. concinna* s. s. In his taxonomic study of the palaearctic *Chaetocnema*, Heikertinger (1951) had noted that one specimen from Semirech'ye was different from the typical specimen of *Ch. concinna* s. s. in aedeagal structure, but he believed this to be some sort of intraspecific variation. Lubischew (1962) regarded this form as a distinct species (*Ch. heptapotamica*), which is apparently found only in Semirech'ye (southern Kazakhstan and Kirghiz). He was convinced that another form, occurring from the Far East to the Vosges Mountains in France, was also a distinct species. This taxon (*Ch. heikertingeri*) is broadly sympatric with *Ch. concinna* s. s., which has a more limited range. The distinctness of the three species was substantiated by biological information and statistical analysis. The discriminant function of Fisher (1936) was used to compare and to distinguish the three species. The characters chosen by statistical means were graphically presented, following the procedure of Romanovsky (1925). For any two of three species of *Chaetocnema*, a pair of discriminant functions, each based on three characters, provided excellent discrimination. Lubischew (1963) finally described these two species, *Ch. heikertingeri*

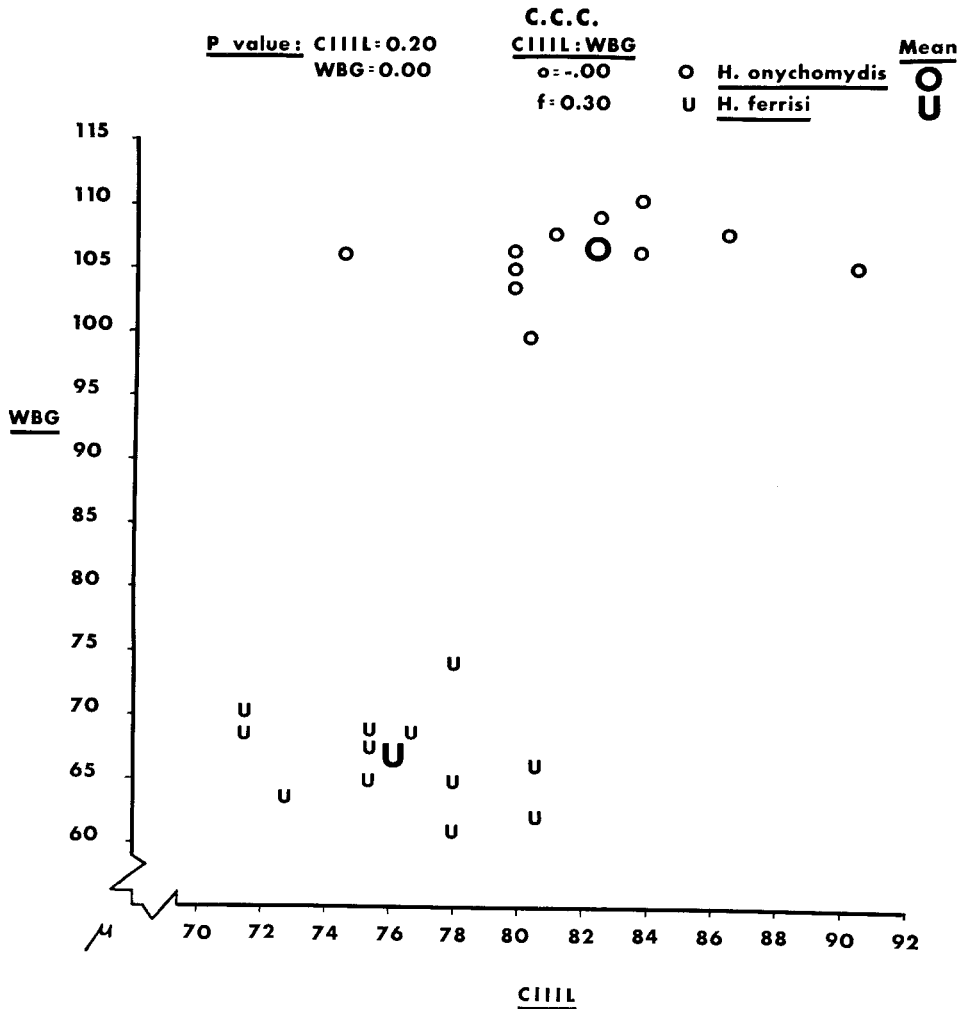


FIG. 7. Scatter diagram showing discrimination between females of two species of *Hoplopleura*.

Lubisch. and *Ch. heptapotamica* Lubisch., by means of measurements and ratio of various structures, geographical distribution, drawings of the aedeagus and fore tarsi, and scatter diagrams with correlation ellipses.

DuPrav (1964, 1965) studied the divergence and relationship of honeybees from 14 geographical areas in the world, using discriminant functions. This paper illustrates very well the discriminant analysis as a taxonomic "screening" procedure.

*A posteriori investigation of taxonomic*

*characters.*—Cryptic species are usually recognized and confirmed by various biological attributes other than morphological characters. However, in most cases, once cryptic species are recognized further study reveals morphological differences that are distinct enough to be used for identification. Many cryptic species initially discovered and distinguished through biological attributes are now distinguished by diagnostic morphological characters, as in the *Drosophila pseudoobscura* complex and the *Anopheles maculipennis* complex.

In 1935 Graham originally pointed out that the pine-feeding form of the spruce budworm, *Choristoneura fumiferana s. l.* (Lepidoptera: Tortricidae), was biologically distinct from the spruce-balsam form and could be considered specifically distinct. Because of their economic importance these two forms were intensively studied, and many differences in biological traits between the two forms were revealed. Freeman (1953) described the pine-feeding form as *C. pinus* and indicated that it could be distinguished by ground color, wing pattern, wing expanse, and male genitalia.

Many cryptic species among sound-producing ensiferan Orthoptera have been recognized by studies of their calling songs, life histories, and the structure of the stridulatory apparatus. Studies of the stridulatory apparatus of cryptic species which are in general morphologically identical have revealed a few useful morphological characters (Walker, 1964).

After discrimination between the adults of *Hoplopleura hesperomydis* and *H. ferrisi* was established statistically, *a posteriori* investigation of the taxonomic characters of the adults revealed some good qualitative discriminatory characters for both species, e.g., head setae, thoracic sternal plate, abdominal paratergal plates, and genitalia of both sexes (Kim, 1965). At the same time Kim found three new species and one new subspecies in the *H. hesperomydis* complex. For comprehensive summaries of examples of cryptic species, refer to Dobzhansky (1937), Brown (1959), and Mayr (1942, 1963).

There are many cryptic species which cannot at present be diagnosed on the basis of morphological characters but which are distinct in other biological attributes, e.g., biochemical components, chromosomes, bioluminescence, behavior, life history, calling songs, host preference, and others. Once the species is recognized, systematists must attempt to provide the most convenient, simple, and easily observable diagnostic characters to facilitate subsequent identification. Since morphological traits

are the easiest taxonomic characters to observe and use, systematists should try to provide morphological characters or a combination of such characters, or some measure derived from morphological observations for diagnostic purposes. The technique of obtaining diagnostic characters based on measurements of morphological characters, as described in Kim, Brown, and Cook (1963) and in the first part of this paper, is outlined and discussed below.

1. The morphological characters to be measured are selected on the basis of previous taxonomic experience, as potentially good discriminators with little correlation within species. Previous experience may range from hunches to extensive correlation matrices on the groups under study.

2. The sample size for each population or species (species will be used in the following discussion) for a study of this kind is determined by previous studies, such as a pilot study of the material.

3. The mensuration system to be used must be standardized.

4. The degree of reliability of the measurements should be assessed.

5. Measurements are taken with extreme care not to introduce artificial differences between groups.

6. The mean and standard deviation for each character in all samples under study are computed.

7. Homogeneity of specimens of the same species from various sources is tested by using the test of homogeneity or analysis of variance.

8. Species are tested pairwise on each variable individually, and when possible on all variables simultaneously, to determine the weight of evidence for considering the species distinct.

9. The proportion overlap or misidentified ( $P$ ) is computed for each character for each pair of species, assuming particular characters alone were used to classify the specimens into one of two species in question.

10. The intercharacter correlation is

computed for each character for each sample of the species under study.

11. The best discriminatory characters are chosen to use as the diagnostic characters for the species in question on the basis of low probability of misidentification and low intercharacter correlation.

12. If such individual diagnostic characters are sufficient to identify or discriminate the species in question, they can be used both in the diagnosis and as part of the species description, e.g., Lubischew (1963). However, if any of these individual characters does not provide sufficient discrimination among species, it can be combined with others to give better discrimination, as demonstrated by Stone (1947), Reed and Reed (1948), Bigelow and Reimer (1954), Lubischew (1962), and Kim, Brown, and Cook (1963).

If characters give a reasonable discrimination between species with considerable overlap, they can be combined to yield a species index of morphological origin, e.g., Reed's wing index (Reed, Williams, and Chadwick, 1942), special index (Reed and Reed, 1948), and the character index (Kim, Brown, and Cook, 1963). Finally, if good discrimination is difficult to achieve, all characters can be employed in the discriminant function of Fisher (1936). In such cases, validation on further samples of specimens is imperative since such validation shows a marked decrease in the apparent discriminating ability of the linear discriminators. For the theory and computation of discriminant function or analysis, refer to Rao (1952), Goulden (1952), and Schultz and Goggans (1961).

#### *Conclusion and Summary*

In this paper discrimination of adults of three louse species, *Hoplopleura hesperomydis* (Osborn), *H. onychomydis* Cook and Beer, and *H. ferrisi* Cook and Beer is investigated. The mensuration system for *Hoplopleura* is standardized. The data are analyzed using character means, standard deviations, probability of misidentification,

intercharacter correlation, and discriminant functions for each species.

The three louse species are more or less distinctly discriminated and distinguishable, and differences among louse populations within a species caused by factors such as different host species, different subspecies of same host species, and host animals from different localities are negligible. The characters used for diagnosis of species are, for males, TBL and TSW; for females, TSPL and TSPW. If two characters did not provide sufficient discrimination among three species when used simultaneously, another pair of characters with better discriminating power was used to discriminate a particular pair of species, and the discriminant functions were computed for each of six discriminations (between the species, pairwise, for the three species) to confirm the species discrimination.

Methods of selecting taxonomic characters are demonstrated. The tasks of systematists and the nature of the species are discussed. Literature pertaining to statistical means of recognizing species is reviewed. The means of recognizing species, and of their subsequent identification with *a posteriori* taxonomic characters, once the species is recognized, are discussed and outlined.

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