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Field Identification of Peromyscus maniculatus and P. leucopus in Maryland: Reliability of Morphological Characteristics

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In western Maryland, two external criteria, the ratio tail length/head and body length in conjuction with body weight, correctly identified 93.2% of adult *Peromyscus maniculatus* and *P. leucopus* to species, without resourse to subjective characteristics. Multiple stepwise discriminant function analysis was also used with several morphological variables to differentiate between the two species. It was able to classify the two species correctly in 95.1% of all cases. No morphological characteristic by itself or in combination was found to reliably identify these two species 100% of the time.

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1. INTRODUCTION

In some areas where deer mice (*Peromyscus maniculatus* Wagner, 1845) and white-footed mice (*P. leucopus* (Rafinesque, 1818)) are sympatric, "... individual specimens are sometimes referred to the correct species only with considerable difficulty ..." (Hall, 1981: 685). This is the situation in much of southern New England (Walters, 1962). Criteria commonly used to distinguish between these species are often quite subjective. *Peromyscus maniculatus* in New England often have a tail pencil, sharply bicolored tail, and grayish pelage that is soft and luxuriant, with only a slight middorsal stripe. Conversely, *P. leucopus* have a more unicolored tail, seldom with a pencil, and more reddish pelage, seldom soft and luxuriant, with a well-defined middorsal stripe (Choate, 1973). *Peromyscus leucopus* have also been described as more "bug-eyed", and excitable when handled than are *P. maniculatus* (Aquadro & Patton, 1980.) Such subjective criteria may lead to misidentification if field personnel are inexperienced, or in areas where differences in these characteristics are minimal between species or variable within species.

In the southern Appalachian region of western Maryland, we found many subjective criteria quite variable and of little value in distinguish-

ing between *P. m. nubiterrae* and *P. l. noveboracensis* (see Paradiso, 1969). Our goal in this study was to find one, or a combination of, objective, external, morphological characteristics that could be used in the field to distinguish between *P. maniculatus* and *P. leucopus* without recourse to subjective characteristics, or relatively expensive and time consuming laboratory techniques such as electrophoresis or karyotyping.

2. MATERIAL AND METHODS

Most of the specimens were collected from the Frostburg Reservoir area, Garrett County, Maryland (approx. 39°42'N. lat., 79°00'W. long.). Additional specimens were obtained from forest, open field or residential areas throughout western Maryland in Garrett and Allegany counties. Garrett County averages about 830 m above sea level, and Allegany County about 670 m. Predominant forest overstory vegetation in Garrett County included sugar maple (*Acer saccharum*), red and white oaks (*Quercus rubra, Q. alba*), and black cherry (*Prunus serotina*). Eastern hemlock (*Tsuga canadensis*) was also an important species, especially in bottomland areas near streams (Gates and Harman, 1980). Forests in Allegany County are dominated by chestnut (*Q. prinus*), white and black oaks (*Q. velutina*) and several hickories (*Carya ovata, C. tomentosa, C. glabra*). All specimens were collected by snap-trapping from October to November from 1980 through 1982.

Most specimens were kept frozen at 0°C, some for periods of up to 18 months, until analyzed. Specimens were then thawed, weighed to the nearest 0.1 g, and the following standard measurements taken to the nearest mm: total length, tail length, ear length, and hind foot length. Two ratios were calculated: tail length/head and body length; and (body weight/total length) × 10. Amount of tail penciling was classified as distinct, semi-distinct, semi-indistinct, or indistinct. Tails were classified as either sharply bicolored with a dark dorsal stripe, or weakly bicolored with light dorsal and ventral surfaces. Pelage color was classified as either reddish or grayish, and the mid-dorsal stripe as distinct or indistinct. Age (adult, subadult or juvenile) was based on body weight and pelage characteristics (Dice & Bradley, 1942; Layne, 1968) for each species. For consistency, all body measurements, subjective criteria and age estimations were made by one of us (GAF).

Several drops of distilled water were then added to the mouth of each specimen. This solution was collected in small test tubes. Species identification was based on electrophoresis of salivary amylase, using the general procedures of Aquadro & Patton (1980) with the following modifications. Electrophoresis was conducted with both 5—20% continuous gradient gels (Isolab-Type II) and 5% polyacrylamide gels. Samples were run for 4 hours at 8°c in a Isolab vertical chamber using the Tris-citric acid electrode buffer (pH 8.7) described by Ridgeway *et al.* (1970). Samples of 10 µl were placed in plastic insert sample wells. Overlays consisted of 1% soluble potato starch, 3% agar, and 0.02% sodium chloride. The overlays were rigid enough to be removed after incubation for staining.

A small amout of muscle tissue was collected from about one-half of the individuals. Standard horizontal starch-gel electrophoresis (as described by Allendorf *et al.* 1977). was used to routinely survey muscle proteins. Allozyme systems analyzed included aspartate aminotransferase, α-glycerophosphate dehydrogenase, adenylate kinase, creatin phosphokinase, esterase, glucose-6-phosphate dehydrogenase, isocarate dehydrogenase, lactate dehydrogenase, malate dehydrogenase, malic

enzyme, phosphoglucose isomerase, phosphoglucomutase and sorbitol dehydrogenase in addition to the salivary amylase.

Data were analyzed using standard programs from the Statistical Package for the Social Sciences (Nie *et al.*, 1975). Statistical significance was considered to be $P \leqslant 0.05$.

3. RESULTS

A total of 119 *Peromyscus* was identified electrophoretically. Species were easily resolved by two distinct amylase variants; a "fast" band (*P. leucopus*) or a "slow" band (*P. maniculatus*). The variants observed probably correspond to *Amy*-1^{100} and *Amy*-1^{76} reported by Aquadro & Patton (1980). The substantial differentiation between these two allozymes was not affected by the gel types utilized in this study or the length of time samples were frozen. Although gene frequency differences between the species were found with some muscle proteins, no alleles, at these loci unambiguously identified the two species. Of the 119 *Peromyscus*, 64 adult or subadult *P. maniculatus* and 39 *P. leucopus* were included in further analyses. Juveniles (N=16) were excluded because of small sample size and differences in the morphological characteristics of subadults and adults, and juveniles.

Based on Students *t* tests, there were statistically significant differences between the two species in all body measurements except hind foot length. However, because of variation in body measurements and resultant overlap between species (Table 1), any given characteristic alone was considered to be diagnostic.

Considerable ambiguity also was noted between species in subjective morphological characteristics. Of 64 *P. maniculatus*, 54 (84.4%) had the expected distinct penciling, 9 had semi-distinct penciling, and 1 had semi-indistinct penciling. However, only 26 of 39 *P. leucopus* (66.7%) had the expected indistinct penciling, 6 had semi-indistinct penciling, and 7 had semi-distinct penciling. Thus, while 80 subadult or adult specimens had the "correct" tail penciling, 23 specimens (22.3% of all subadults and adults) were ambiguous with regard to this characteristic. Likewise, whereas a sharply bicolored tail was noted in 59 (92.2%) *P. maniculatus*, this characteristic was also seen in 12 (30.8%) of the *P. leucopus*.

Conversely, the expected reddish pelage color was seen in all subadult and adult *P. leucopus*. Hovewer, reddish pelage also occurred in 30 of 64 (46.9%) *P. maniculatus*. Thus, while gray pelage was a diagnostic characteristic for *P. maniculatus*, reddish pelage was of little value in species determination. Finally, the expected distinct middorsal stripe

Table 1

Comparison of body measurements between *Peromyscus maniculatus* and *P. leucopus* from Allegany and Garrett counties, Maryland.

Mean body measurement	*P. maniculatus* (N=64)	*P. leucopus* (N=39)	*t* value
Body weight (g)	16.8 (2.3)[a] (12.2—22.1)[b]	20.8 (3.2) (14.1—30.5)	6.91[3]
Total length (mm)	166.7 (9.7) (149—193)	160.7 (9.5) (140—181)	3.01[2]
Tail length (mm)	82.6 (6.7) (69—102)	71.5 (5.5) (59—81)	8.80[3]
Head and body length (mm)	83.9 (5.3) (69—100)	89.2 (6.0) (76—110)	4.65[3]
Ear length (mm)	17.0 (1.1) (14—20)	16.5 (0.9) (15—19)	2.48[1]
Hind foot length (mm)	20.5 (0.8) (18—22)	20.6 (0.8) (18—22)	0.49
Tail length H & B length	0.99 (0.1) (0.79—1.17)	0.80 (0.1) (0.64—0.94)	11.67[3]
(Body weight/total length) ×10	1.00 (0.1) (0.77—1.33)	1.29 (0.1) (1.01—1.68)	11.20[3]

[a] Standard deviation. [b] Range. [1] $P<0.05$; [2] $P<0.01$; [3] $P<0.001$.

was noted in 37 (94.9%) *P. leucopus*. Contrary to expectations, it was also evident in 43 (67.2%) of the *P. maniculatus*.

With only one exception, adult and subadult specimens with tail length/head and body length ratios $\geqslant 0.91$ were *P. maniculatus*, and specimens with a ratio $\leqslant 0.81$ were *P. leucopus*. There was considerable species overlap between these values. In our sample, however, the species of adults or subadults with tail length/head and body length ratios between 0.82 to 0.90, generally could be determined on the basis

Table 2

Summary of multiple stepwise discriminant function analysis conducted on *Peromyscus maniculatus* and *P. leucopus* from Allegany and Garrett counties, Maryland.

	Discriminant Function I
Eigenvalue	2.519
Relative percentage of eigenvalue associated with the function	100.0
Canonical correlation	0.846
Chi-square statistics	125.18[a]
(Degrees of freedom)	(3)
Pooled within-groups correlations between discriminant function and discriminating variables:	
Tail length/head and body length	0.732
(Body weight/total length) ×10	−0.702
(Head and body length	−0.292

[a] $P<0.001$

of body weight. Within the 0.82—0.90 ratio range, all specimens (N=7) with body weights less than 18.0 g were *P. maniculatus;* all 10 *P. leucopus* had body weights $\geqslant$18.0 g, as did 2 *P. maniculatus.* Pregnant females were excluded.

Four body measurements and the two calculated ratios were used in a multiple stepwise discriminant function analysis (SWDA). Prior to analysis, all variables were tested for normality. Two highly correlated ($r\geqslant 0.7$) variables, body weight and total length, were deleted from the analysis. The assumption of homogeneity of group covariance matrices was tested and found to be non-significant (Box's M=11.84, $P>0.05$). The discriminant function model derived from the analysis correctly classified all 39 adult and subadult *P. leucopus* and 59 of 64 adult and subadult *P. maniculatus* (92.2%) for a total correct classification of 95.1% ($\chi^2=84.16$, d.f.=1, $P<0.001$). The three discriminating variables selected by SWDA and their associated correlation coefficients (Table 2) demonstrated that the ratio of tail/head and body length and the ratio of (body weight/total length)$\times$10 were of most value in differentiating between *P. maniculatus* and *P. leucopus.* Two group centroids for *P. leucopus* and *P. maniculatus* along the derived discriminant function were at — 2.01 and 1.23, respectively.

4. DISCUSSION

The well-documented regional variability in morphological characteristics of *P. maniculatus* and *P. leucopus* (Waters, 1963) was apparent in this study. In New England, *P. maniculatus* generally have tail length/head and body ratios $\geqslant$1.00 (Choate, 1973). In western Maryland, we found only 31 of 64 (48.4%) adult or subadults had a ratio $\geqslant$1.00. Generally, however, *P. maniculatus* did have relatively longer tails than *P. leucopus,* and this characteristic was of partial value in species determination. Pelage color, tail penciling, and bicoloration were also quite variable within-species and of limited value in species identification.

Although we were unable to determine a univariate characterictic to differentiate *P. maniculatus* from *P. leucopus,* the use of the tail length/head and body length ratio correctly referred 79 of 103 adults or subadults (76.7%) to species. Of 22 individuals in the "overlap zone" (a ratio of 0.82—0.90), 17 could then be correctly referred to species by body weight. Thus, excluding two pregnant females, 96 of 103 subadults or adults (93.2%) were correctly identified based on two measurements and body weight, without recourse to subjective characteristics. However, for analyses made during or immediately following the reproductive

season, some subjectivity may remain in distinguishing between juveniles and subadults, or in positive identification of pregnant females.

The discriminant function model based on head and body length and two ratios successfully referred 98 of 103 (95.1%) adults and subadults to the correct species. Choate (1973) developed a linear discriminant function model based on 11 skull measurements and used it to differentiate between *P. maniculatus* and *P. leucopus* in New England. Although neither such analyses resulted in a "field" identification technique, the one based on skull measurements has the obvious drawback of sacrificing the specimen. The discriminant function derived in this study resulted in about a 2% greater predictive ability than the two-step ratio-body weight field procedure noted previously.

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POLOWA IDENTYFIKACJA *PEROMYSCUS MANICULATIS* I *P. LEUCOPUS* W MARYLAND: RZETELNOŚĆ CHARAKTERYSTYKI MORFOLOGICZNEJ

Streszczenie

Celem niniejszej pracy było znalezienie obiektywnej, opartej na wyglądzie zewnętrznym zwierzęcia, charakterystyki morfologicznej, która używana w warunkach polowych, pozwalałaby na rozdzielenie dwóch sympatrycznych gatunków *P. maniculatus* i *P. leucopus*.

Badania prowadzono w zachodnim Maryland. Uwzględnienie takich cech jak stosunek długości ogona i długości ciała do ciężaru ciała (Tabela 1) dawało poprawną identyfikację, 93,2% dorosłych *P. maniculatus* i *P. leucopus*. W celach porównawczych zastosowano również wielokrotną analizę dyskryminacyjną (Tabela 2) kilku morfologicznych zmiennych. Tutaj dokładność rozdziału wyniosła 95,1%. Dokładności 100% przy użyciu charakterystyki morfologicznej nie udało się osiągnąć.