

Mensural discrimination between sympatric *Peromyscus leucopus* and *P. maniculatus* in southern Illinois

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Sternburg J. E. and Feldhamer G. A. 1997. Mensural discrimination between sympatric *Peromyscus leucopus* and *P. maniculatus* in southern Illinois. Acta Theriologica 42: 1-13.

A total of 383 *Peromyscus* was collected from southern Illinois to determine morphological characteristics useful in identifying individuals as either *P. leucopus* (Rafinesque, 1818) or *P. maniculatus* (Wagner, 1845). Polyacrylamide gel electrophoresis of salivary amylase was used to positively identify all specimens. No univariate morphological character accurately discriminated between the two species because of a high degree of intraspecific variation. Stepwise discriminant function analysis of external characters correctly classified 97.9% of subadults to species. The most important external character was the tail length/body length ratio. This ratio was also the most important factor in discrimination of adults; the function correctly classified 98.6% of individuals. Considering skull measurements of adults, 9 cranial characters were needed to differentiate between the two species, with a correct classification of 98.9%. For old adults, all specimens were classified correctly using 5 cranial characters. There was no fast, easy, accurate method to discriminate between these species 100% of the time in the field.

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Key words: *Peromyscus leucopus*, *Peromyscus maniculatus*, discriminant function, electrophoresis, morphometrics

Introduction

Mice of the genus *Peromyscus* (Gloger, 1841) are one of the most widely distributed rodents in North America. Sympatric occurrences of *Peromyscus* are common as geographic ranges of many species overlap. Where morphologically similar-looking species coexist, proper identification is often difficult. In many instances, mensural and cranial characteristics, with subjective morphological traits such as pelage coloration and tail penciling, are used to differentiate between these species. However, for some sympatric species of *Peromyscus*, ranges of mensural characteristics overlap, making it difficult to assign certain individuals to species (Engstrom *et al.* 1982, Thompson and Conley 1983, Allard *et al.* 1987).

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Several methods have been used to identify individual *Peromyscus* to species. These include adrenal weight (Christian 1967), size of the calcaneum (Stains 1959), red blood cell immune agglutination (Moody 1941), karyotype (Hsu and Arrighi 1966, Pathak *et al.* 1973), and electrophoresis of salivary amylase (Aquadro and Patton 1980). Several of these methods require sacrificing the animal and are not viable for many ecological or behavioral studies. Also, they are expensive, time consuming, require equipment which may not be readily available, or can not be done in the field.

Several researchers have differentiated between similar-looking, sympatric species of *Peromyscus* using discriminant function analyses of external or cranial measurements (Choate 1973, Choate *et al.* 1979, Stromberg 1979, Feldhamer *et al.* 1983, Thompson and Conley 1983, Allard *et al.* 1987). While this method is valid for identifying individuals from sympatric *Peromyscus* populations, results differ among geographic areas. Also, significant age variation occurs for many external and cranial characters.

In southern Illinois, the deer mouse *P. maniculatus bairdi* (Wagner, 1845), and the white-footed mouse *P. leucopus leucopus* (Rafinesque, 1818) are sympatric and often difficult to differentiate. Although sympatric, they often occur in different habitat types (ie allotopic). Deer mice are usually found in relatively open areas, whereas white-footed mice primarily inhabit forested areas (Schwartz and Schwartz 1981, Mumford and Whitaker 1982, Hoffmeister 1989). Throughout the midwestern United States, however, both of these species sometimes occur together in the same local area (Whitaker 1967, Hansen and Warnock 1978, Kantak 1983, Clark *et al.* 1987).

Skulls of *P. leucopus* and *P. maniculatus* also are difficult to differentiate to species. Although Hoffmeister (1989) discriminated skulls of adult specimens of the two species from Illinois based on several measurements, Mumford and Whitaker (1982) felt there was no good character for discriminating skulls of these two species in Indiana.

The objectives of this study were: (1) to positively identify samples of *P. maniculatus* and *P. leucopus* from southern Illinois using electrophoresis of salivary amylase; (2) to determine if these specimens could be discriminated by subjective morphological traits; (3) to determine diagnostic external or cranial characters for distinguishing these species; (4) to derive a classification function, using discriminant analysis of external characters, to identify these species in the field; and (5) to derive a classification function, using discriminant analysis of cranial measurements, to identify skulls of both species.

Materials and methods

We examined 383 *Peromyscus* for this study; 139 specimens were taken from 14 counties throughout southern Illinois during 1985–1986. An additional 244 specimens were collected from

October 1988 through March 1991. Snap traps were baited with peanut butter and set in favorable habitats. Specimens were returned to the lab and frozen at -70°C until analyzed.

Morphological analyses

Measurements (mm) taken included: (1) body mass (nearest 0.1 g), (2) total length (tip of nose to last tail vertebra), (3) tail length (sacrum to last tail vertebra), (4) hind foot length (heel to tip of longest claw), (5) ear length (notch to tip), and (6) body length (total length minus tail length). Two ratios were calculated from these data: RAT1: tail length/body length, and RAT2: (body mass/total length) \times 10.

Three pelage characteristics often used to identify *Peromyscus* (tail penciling, tail bicoloring, and mid-dorsal stripe) were evaluated as distinct, semi-distinct, semi-indistinct, or indistinct for the 244 individuals trapped between 1988 and 1991. In addition, pelage color (brown, reddish brown, grayish brown, brownish black, or predominantly gray) was evaluated.

The reproductive condition of each specimen was determined prior to skull removal. Females were recorded as pregnant if their abdomens were swollen and/or pups were present *in utero*, lactating if the nipples were enlarged, or nonbreeding if neither of these conditions was observed. Males were recorded as breeding (descended testes) or nonbreeding (abdominal testes) (Wolff 1985).

Skulls were removed and cleaned by dermestid beetle larvae. Dial calipers were used to take 14 cranial measurements (Choate 1973, Thompson and Conley 1983, Hoffmeister 1989) to the nearest 0.05 mm. Each measurement was taken three times (by JES) and the average was recorded. Skull measurements were: (1) greatest length (GLS – from anterior point on nasals to posterior point on occiput), (2) zygomatic breadth posterior (ZBP – greatest breadth across posterior portion of the zygomatic arches), (3) zygomatic breadth anterior (ZBA – greatest breadth across anterior portion of the zygomatic arches), (4) interorbital constriction (IC – least breadth of interorbital region), (5) greatest rostral breadth (GRB – breadth across rostrum just anterior to infraorbital foramina), (6) basonasal length (BL – anterior inferior border of foramen magnum to anterior point on nasals), (7) length of bony palate (LBP – posterior margin of incisive foramina to midpoint of posterior margin of palate), (8) total length of toothrow (TLT – anterior crown surface of upper incisor to posterior crown surface of corresponding upper third molar), (9) length of maxillary toothrow (LMXT – anterior crown surface of upper first molar to posterior crown surface of upper third molar), (10) breadth across molars (BAM – breadth of palate and crown surfaces of the third upper molars), (11) pterygoid breadth (PB – least breadth across pterygoids), (12) cranial depth (CD – from dorsal surface of brain case to ventral surface of auditory bullae, at 90° angle to GLS), (13) rostral length (RL – from anterior point of nasal bones to anterior edge of zygomatic arches), and (14) nasal length (NL – from anterior point of nasal bones to posterior point, taken along midline). Specimens were assigned to one of four age classes (juvenile, subadult, adult, or old adult) based upon pelage and molar wear as described by Cockrum (1954), Choate *et al.* (1979), and Wolff (1985).

Electrophoretic analysis

Prior to removing the skull from each individual a sample of saliva was taken by positioning the mouse on its back and placing several drops of distilled water into its mouth. After five minutes, the distilled water was pipetted out of the mouth and into a labeled plastic centrifuge tube (Aquadro and Patton 1980, Feldhamer *et al.* 1983).

Electrophoresis of saliva samples was carried out in 5% polyacrylamide ($16 \times 18 \times 1.5$ mm) using a vertical slab gel electrophoresis unit, Model SE 600, with a water cooled heat exchanger (Hofer Scientific Instruments, San Francisco, CA). See Sternburg (1992) for details of the electrophoretic procedure. Salivary amylase electromorphs were identified based upon percent mobility of the band relative to the common electromorph of *P. leucopus*, chosen as *Amy-1¹⁰⁰* (Aquadro and Patton 1980). Specimens with amylase variants of high mobility were designated *P. leucopus*, and those with lower mobility were designated *P. maniculatus* (Aquadro and Patton 1980).

Data analyses

External and cranial character sets were analyzed separately. Specimens were grouped by age class and species, and standard statistics (mean, range, and standard deviation) were computed for each character and ratio using the Statistical Package for the Social Sciences, release 4.1 (Norusis/SPSS Inc. 1990). No further analyses were performed on mensural characters of juveniles due to small sample sizes.

Secondary sexual variation is considered non-significant for *Peromyscus* in Illinois (Hoffmeister 1989). We tested this hypothesis using *t*-tests with $\alpha = 0.05$. Only one cranial character (zygomatic breadth-posterior, for adult *P. maniculatus*) and one external character (body length, for adult and old adult *P. maniculatus*) showed significant sexual variation. Therefore, to maximize sample sizes, males and females were grouped together.

Variation between subadults, adults, and old adults within each species, for each character set, was examined using analysis of variance with $\alpha = 0.05$. Significant variation was found between subadults and the other age classes within both species; further analyses were performed separately on subadults. Significant variation in cranial measurements also was found between adults and old adults within each species. Thus, five data sets were analyzed for each species: two data sets for external measurements – subadults and adults/old adults combined; and three sets for cranial measurements – subadults, adults, and old adults.

All data sets were examined for missing values, outliers, normality, linearity, multicollinearity, singularity, and homogeneity of variance-covariance matrices using histograms, normal plotting, bivariate scatterplots, and residual plotting. Additionally, the Shapiro-Wilks' statistic for normality was examined using PROC UNIVARIATE, with $\alpha = 0.1$. Non-normal characters were further evaluated using *z*-values. The *z*-values were calculated using the skewness and standard error of skewness of each character and evaluated at $\alpha = 0.01$ (Tabachnick and Fidell 1989). Multivariate outliers were determined using SPSS REGRESSION, and the Mahalanobis distance, using χ^2 with degrees of freedom equal to the number of predictor variables, at $\alpha = 0.001$. Any individual with a Mahalanobis distance greater than the χ^2 value was considered a multivariate outlier (Tabachnick and Fidell 1989). Homogeneity of variance-covariance matrices was evaluated using Box's *M* statistic at $\alpha = 0.05$ (Tabachnick and Fidell 1989). Differences in character means between species were tested for each of the five data sets using one-way ANOVA's with $\alpha = 0.05$.

Stepwise discriminant function analysis, using the DISCRIM program of SPSS, was performed on each of the five sets of external and cranial measurements to predict group (species) membership for each specimen, and to reduce the number of characters needed to discriminate between the two species to as few as possible (Allard *et al.* 1987, Tabachnick and Fidell 1989). The criteria used for entering and removing variables from the stepwise discriminant function was minimization of Wilks' lambda, with an *F* to enter and an *F* to remove of 1.00 (Norusis/SPSS Inc. 1990). The linear function produced allowed a discriminant score for each specimen to be calculated, which was used to classify each individual.

Skulls often were damaged during capture, precluding our taking certain measurements. Because discriminant function analysis will delete any case missing one or more of the predictor variables, mean values for each age group and species were substituted for missing values (Tabachnick and Fidell 1989) for both adult and old adult data sets. Specimens missing 5 or more of the 14 cranial characters were not used in analyses.

Specimens positively identified to species using electrophoresis were assigned *a priori* and used to form the stepwise discriminant function. Known specimens were then reclassified using the discriminant function to obtain the percentage classified correctly. Specimens for which a saliva sample was unavailable, or electrophoresis of saliva proved inconclusive, were treated as unknowns, and only classified by the discriminant function.

Results

Electrophoresis and species assignment

Salivary amylase samples from 375 *Peromyscus* were examined electrophoretically. Four amylase electromorphs (bands) were observed. These bands probably corresponded to four of the seven variants Aquadro and Patton (1980) found, and based on percent mobility were identified as *Amy-1*⁷⁶, *Amy-1*⁸⁵, *Amy-1*¹⁰⁰, and *Amy-1*¹⁰⁹. Evans *et al.* (1977) noted three amylase electromorphs of *P. maniculatus*, of which their "A" and "B" electromorphs probably correspond to *Amy-1*⁷⁶ and *Amy-1*⁸⁵, respectively. Further reference to electromorphs will use the nomenclature of Aquadro and Patton (1980).

The bands with slower mobility, *Amy-1*⁷⁶ and *Amy-1*⁸⁵, are unique to *P. maniculatus*, and those with faster mobility, *Amy-1*¹⁰⁰ and *Amy-1*¹⁰⁹, are unique to *P. leucopus*. The most common variants found in this study for *P. leucopus* and *P. maniculatus* were *Amy-1*¹⁰⁰ and *Amy-1*⁷⁶, respectively. This corresponds to the findings of Aquadro and Patton (1980). Based on the observed banding patterns, 143 specimens were identified as *P. maniculatus*, 225 specimens as *P. leucopus*, and 7 as unknowns. Unknown specimens showed either no amylase activity or an odd banding pattern that indicated protein breakdown.

Subjective morphological characters

Pelage characteristics were evaluated for 127 adult and subadult *P. maniculatus* and 106 adult and subadult *P. leucopus*. Juveniles were excluded from this analysis due to the differences between juvenile pelage characteristics and those of subadults and adults.

Tail penciling was slight in *P. leucopus*, with 81.9% of the specimens having indistinct (no long hairs extending past the posterior portion of the last tail vertebra), or semi-indistinct penciling (a few long hairs). Tail penciling was more pronounced in *P. maniculatus*, but still indistinct or semi-indistinct in 45% of the sample.

Only two white-footed mice had sharply bicolored tails, while 55% had indistinctly bicolored tails. This was expected as bicoloredness of tail in white-footed mice is weak (Hoffmeister 1989). This is in contrast to *P. maniculatus*; 19.0% had the expected sharply bicolored tail and approximately 41% were classified as having semi-distinct bicolored tails. As with tail penciling, however, both species were represented in all categories of tail bicoloredness.

The mid-dorsal stripe in *P. maniculatus* was not as prominent as expected. No deer mouse had a distinct mid-dorsal stripe, and only 30% had a semi-distinct stripe. Most *P. leucopus* had an indistinct mid-dorsal stripe; however, 15 individuals had a semi-distinct mid-dorsal stripe, while 1 had a distinct mid-dorsal stripe.

Pelage of *P. leucopus* was generally lighter in color and more reddish brown than that of *P. maniculatus*, which was darker and had more of a grayish hue. However, within-species variation was pronounced; 15.8% of *P. maniculatus* were

reddish brown, and 17.9% of *P. leucopus* were grayish brown, brownish black, or predominantly gray.

External morphometrics - Univariate analyses

Subadults. After eliminating specimens because of missing values or outliers, external measurements were taken on 23 *P. maniculatus* and 24 *P. leucopus*. The means of subadults were significantly different between the two species for five of the eight characters. However, due to individual variation, the ranges of four of these characters overlapped, making them unreliable diagnostically. One variable may be diagnostic for the two species as the ranges did not overlap: RAT1 (tail length/body length) for *P. maniculatus* was 0.51–0.70, for *P. leucopus* 0.72–1.01. However, this ratio almost overlapped; with larger sample sizes, RAT1 may not be diagnostic.

Table 1. Univariate external characters of adult *Peromyscus* collected from southern Illinois, 1985–1991. All values in mm except mass and the ratios RAT1 and RAT2. * - $p \leq 0.001$, ^a Standard deviation, ^b $df = 1, 270$.

Character	<i>P. maniculatus</i> (n = 104)		<i>P. leucopus</i> (n = 168)		F-value ^b
	Mean (SD) ^a	Range	Mean (SD)	Range	
Mass (g)	20.3 (3.5)	14.5–29.7	21.2 (3.6)	14.5–32.4	4.18
Total length	140.9 (7.4)	124.0–158.0	161.3 (10.2)	136.0–185.0	313.00*
Body length	86.0 (4.6)	75.0–97.0	89.9 (5.5)	74.0–101.0	35.71*
Tail length	54.9 (4.0)	44.0–63.0	71.4 (6.5)	54.0–86.0	536.70*
Hind foot length	18.1 (0.8)	16.0–20.0	20.3 (0.8)	18.0–22.0	541.80*
Ear length	16.2 (1.4)	12.0–19.0	17.4 (1.3)	14.0–20.0	59.23*
RAT1	0.64 (0.04)	0.52–0.75	0.8 (0.07)	0.60–0.99	429.20*
RAT2	1.44 (0.23)	1.03–2.21	1.31 (0.20)	0.96–2.05	23.18*

Adults. Sample sizes for external measurements for adult *P. maniculatus* and *P. leucopus* were 104 and 168, respectively (Table 1). The means of all external characters, with the exception of mass, were significantly different ($p < 0.001$). However, none were diagnostic as the ranges of all characters overlapped.

External morphometrics - Multivariate analyses

Subadults. Stepwise discriminant function analysis was performed on data from 23 *P. maniculatus*, 24 *P. leucopus*, and 7 unknown *Peromyscus* using mass, body length, tail length, hind foot length, ear length, tail length/body length ratio (RAT1), and mass/total length times $\times 10$ (RAT2) as predictor variables. Total length was deleted as a predictor variable because of multicollinearity with both body and tail lengths ($r \geq 0.90$). Unknowns were not used to create the discriminant function, but only in the classification procedure.

Table 2. Standardized and unstandardized coefficients calculated during stepwise discriminant function analyses of external measurements of subadult and adult *Peromyscus* collected from southern Illinois, 1985–1991.

Selected variable	Standardized coefficient	Unstandardized coefficient
Subadults		
RAT1	0.776	12.239
Hind foot length	0.580	0.613
Ear length	-0.344	-0.213
Mass	0.230	0.183
Constant		-19.712
Adults		
RAT1	2.282	37.849
Tail length	-1.698	-0.295
Body length	1.494	0.279
Hind foot length	0.625	0.788
Constant		-48.684

Four predictor variables were used to create the discriminant function, with RAT1 and hind foot length making the greatest contribution (Table 2). RAT1 also had the highest correlation (loading) = 0.82. The eigenvalue of the function (the ratio of between-group to within-group variability) was 4.14, the canonical correlation was 0.897, and Wilk's lambda was 0.195. Thus, variability between species was much greater than within species. The unstandardized discriminant function coefficients (Table 2) were used to calculate discriminant scores of the specimens. The classification function was quite accurate as 46 of the 47 known specimens (97.9%) were classified correctly. The misclassified specimen was one of the smallest subadult *P. leucopus*.

Adult/Old Adults. Stepwise discriminant function analysis was performed on data from 104 *P. maniculatus* and 168 *P. leucopus* and 31 unknown *Peromyscus* using body length, tail length, hind foot length, ear length, and RAT1. Total length was deleted as a predictor variable due to multicollinearity with body length ($r > 0.90$). Mass and RAT2 were deleted as predictor variables due to the significant difference ($p < 0.01$) between means of pregnant and non-pregnant females, and between pregnant females and all other adult *Peromyscus*. Predictor variables were deleted rather than individuals due to the large number of pregnant females; 48% of female deer mice and 24% of female white-footed mice. Also, the sample of adults did not meet the assumption of homogeneity of variance-covariance matrices (Box's $M = 228.26$, $p < 0.001$). Unless sample sizes are large, violation of this assumption can be dealt with either by using equal sample sizes, and the pooled within-group covariance matrix, or by using separate covariance matrices to classify specimens and withholding a percentage (we used 20%) of each sample from the analysis procedure for cross-validation purposes (Tabachnick and Fidell

1989). Both procedures produced essentially the same results; we report only the latter method. Sample sizes used in the analysis were 134 *P. leucopus* and 83 *P. maniculatus*; 34 *P. leucopus* and 21 *P. maniculatus* were withheld for cross-validation.

Of the five predictor variables, all but ear length were selected. The standardized discriminant function coefficients indicated that RAT1 contributed the most to the discriminant function (Table 2). The eigenvalue of the function was 3.59, the canonical correlation was 0.884, and Wilk's lambda was 0.218. As with subadults, variability was greater between species than within species. Again, the classification function was very accurate, with 98.6% of individuals classified correctly. All specimens withheld for cross-validation were correctly classified. Only 3 small white-footed mice were misclassified. These same 3 mice were misclassified by the discriminant function produced using equal sample sizes.

Cranial morphometrics - Univariate analyses

Subadults. Cranial measurements were taken on 22 *P. maniculatus* and 11 *P. leucopus*. Means were smaller for *P. maniculatus* than for *P. leucopus* for all characters, with significant differences ($p < 0.01$) for 12 of 15 measurements. However, no measurement was considered diagnostic due to overlap of ranges for both species.

Adults. Cranial measurements were taken on 79 *P. maniculatus* and 110 *P. leucopus*. Means of all 15 cranial variables of adult specimens were significantly smaller for *P. maniculatus* than for *P. leucopus* ($p < 0.001$). Ranges of all variables overlapped, however, and again were not diagnostic.

Old Adults. Skull measurements were taken from 15 *P. maniculatus* and 35 *P. leucopus*. Again, means of 13 cranial variables were significantly smaller ($p < 0.001$) for *P. maniculatus* than for *P. leucopus*. However, overlap in ranges generally precluded use of univariate cranial characters diagnostically. One variable, total length of toothrow had unique ranges for the two species; with *P. maniculatus* (10.65–11.60 mm) lower than *P. leucopus* (11.65–12.55 mm). This cranial character may be useful in distinguishing between the two species, or it may be an artifact of sample size.

Cranial morphometrics - Multivariate analyses

Adults. A stepwise discriminant function analysis was performed on the cranial measurements of 79 adult *P. maniculatus*, 110 adult *P. leucopus*, and 21 unknown adult *Peromyscus*, using all cranial measurements as predictors except greatest length of skull. This variable was not used because of high multicollinearity with other variables. Nine predictor variables were selected: cranial depth, nasal length, basonasal length, maxillary tooth row length, breadth across molars, greatest rostral breadth, zygomatic breadth anterior, interorbital constriction, and zygomatic breadth posterior. The eigenvalue of the function was 3.82, the canonical

Table 3. Standardized and unstandardized coefficients calculated during stepwise discriminant function analyses of cranial measurements of adult and old adult *Peromyscus* collected from southern Illinois, 1985–1991.

Selected variable	Standardized coefficient	Unstandardized coefficient
Adults		
BL	0.734	1.078
NL	-0.664	-1.501
CD	0.343	1.418
ZBA	-0.307	-0.642
LMXT	0.297	2.198
BAM	0.265	1.542
GRB	0.187	0.951
ZBP	0.183	0.489
IC	0.143	0.876
Constant		-42.750
Old adults		
TLT	0.590	2.370
CD	0.475	1.727
BL	0.320	0.528
NL	-0.316	-0.693
ZPB	0.289	1.094
Constant		-63.331

correlation was 0.890, and Wilk's lambda was 0.208. Again, variability among species was greater than within species.

Basonasal length and nasal length, which were intercorrelated, contributed most to the function and accounted for most of the variability between the species (Table 3). Discriminant scores for each specimen were calculated using the unstandardized discriminant coefficients and these scores were used to classify each specimen. Only two skulls were misclassified, resulting in a correct classification rate of 98.9%. Both misidentified skulls were *P. leucopus* incorrectly classified as *P. maniculatus*. They were more than two standard deviations smaller than the mean for five of the nine variables selected. Both skulls were from individuals initially identified as subadults, however, further analysis of their external characteristics, and amount of molar tooth wear led to classification as adults.

Old Adults. Skulls of 35 old adult *P. leucopus*, 15 old adult *P. maniculatus*, and 4 old adult unknown *Peromyscus* were analyzed. Predictor and grouping variables were the same as those used for adults. Five of 13 predictor variables were selected: total length of toothrow, cranial depth, zygomatic breadth posterior, nasal length, and basonasal length. The eigenvalue of the function was 5.20, the canonical correlation was 0.916, and Wilk's lambda was 0.161. Total length of toothrow and cranial depth accounted for most of the variation between the two species (Table 3). Considering classification results of old adults, all specimens were classified correctly.

Discussion

Species identification of *P. maniculatus* and *P. leucopus* from southern Illinois based on subjective morphological traits such as tail penciling, tail bicoloredness, mid-dorsal striping, and pelage color, is not feasible because of intraspecific variation. Additionally, subjective traits are difficult to score. The difference between "semi-distinct" and "semi-indistinct" for example, is often arbitrary. With one possible exception, no mensural character, when considered singly, was diagnostic for the two species. The possible exception was ratio of tail length/body length of subadults, although larger sample sizes will need to be analyzed to assure that this character is species-specific.

Hoffmeister (1989) reported *Peromyscus* from Illinois can be identified to species using only hind foot length. He suggested deer mice have a hind foot length ≤ 18 mm, while white-footed mice have a hind foot length > 18 mm. Using this criterion alone, 25.8% of deer mice and 2.9% of white-footed mice in this study would have been misidentified. This degree of inaccuracy, especially for deer mice, could be unacceptable in many studies.

Conversely, stepwise discriminant function analysis of external characters successfully distinguished between deer mice and white-footed mice collected in southern Illinois. Subadults were separated based on tail length/body length ratio and hind foot length. For adults, distinguishing characters also included tail length/body length ratio and hind foot length, as well as body length and tail length. Generally, larger values of these characters are associated with white-footed mice, and smaller values with deer mice. Stromberg (1979) reported that ear length and hind foot length, and to a much lesser degree, tail length, were most valuable in discriminating specimens of *P. m. bairdi* and *P. l. leucopus* from southern Wisconsin. He obtained a 100% correct classification rate. In Illinois, ear length generally was not a useful discriminating character.

Tail length and hind foot length are most often referred to when trying to distinguish between the deer mouse and the white-footed mouse. Therefore, it is not surprising that they accounted for much of the discrimination between the two species. The white-footed mouse is considered to be much more arboreal than *P. m. bairdi*. Horner (1954) reported that under laboratory conditions, longer tails and larger feet are adaptations positively correlated with an increased amount of arboreal behavior. Additionally, the tail to body length ratio (RAT1) is greater in semiarboreal species than in terrestrial species. White-footed mice in this study had longer tails, larger feet and a greater tail to body length ratio, than deer mice. Because *P. leucopus* generally occur in woods or brush, increased arboreal adaptations would be advantageous. Conversely, arboreal adaptations in *P. m. bairdi*, usually found where little woody vegetation is present, are not as strongly developed. The woodland subspecies *P. m. gracilis* is similar to *P. leucopus*, and has the same arboreal adaptations, long tail and large feet, and a greater tail to body length ratio (Blair 1950).

Species identification of skulls from white-footed mice or deer mice from southern Illinois probably can not be made consistently on the basis of a single cranial character. Although character means were significantly different between the two species for subadult, adult, and old adult specimens, ranges overlapped within all age groups for virtually all characters. Only one cranial variable had distinct ranges, and then only for one age group – total length of tooththrow for old adults. This may have been an artifact of small sample sizes as only 40 old adult specimens were examined. Ranges of total length of tooththrow overlapped in adults, where the sample size was much larger. However, this character also overlapped in subadults, where the sample size was smaller than for old adults.

Choate *et al.* (1979) examined samples of *P. m. bairdi* and *P. leucopus* in Kansas, and also found that the ranges of total length of tooththrow did not overlap for subadults or old adults. Sample sizes were similar to our study. Thus, it may be possible to distinguish skulls of white-footed mice and deer mice, regardless of age, by total length of tooththrow. Again, larger sample sizes will be needed to confirm this.

Stepwise discriminant function analysis of cranial characters was successful in selecting measurements to accurately distinguish between skulls of white-footed mice and deer mice. Nine and five cranial characters were used to determine species of adult and old adult skulls, respectively. All old adult specimens were classified correctly, based primarily on total length of tooththrow. This was expected as the range of this variable did not overlap for the two species. However, this variable was not useful in separating adult specimens. Basonasal length, nasal length, and cranial depth were most influential in discriminating between skulls of adults. These also were the next most important variables in distinguishing skulls of old adult *Peromyscus*.

Choate *et al.* (1979), using discriminant function analysis of cranial and external measurements together, and evaluating the importance of selected variables based upon the discriminant multipliers, found maxillary tooththrow length, greatest length of skull, basonasal length, and breadth across molars accounted for most of the variation between the two species in Kansas. Basonasal length was the only distinguishing character common to both studies. Choate (1973) used only cranial characters and discriminant function analysis to differentiate between adult *P. leucopus* and *P. m. gracilis*. Based upon discriminant multipliers, he found that maxillary tooththrow length, greatest rostral breadth, and interorbital constriction were the most important characters for discriminating between skulls of these taxa. Considering the unstandardized discriminant variables in the present study, length of maxillary tooththrow was most important in differentiating skulls of adult *P. leucopus* and *P. m. bairdi*. This also is the variable that Choate (1973) and Choate *et al.* (1979) reported as being most influential. For old adults, total length of tooththrow and cranial depth were still the two most influential variables; however, zygomatic breadth-posterior, increased in importance.

Subadult and adult specimens of *P. leucopus* and *P. m. bairdi* in southern Illinois were identified by external measurements with a high degree of accuracy, without sacrificing specimens, using discriminant functions. It is important to correctly identify the age of the specimen or skull in question, as the traits used to discriminate species vary with age. The following equations can be used to accurately identify questionable specimens of *Peromyscus* from southern Illinois:

Subadults: $D = -19.712 + (0.183 \times \text{weight}) + (0.613 \times \text{hind foot length}) + (-0.213 \times \text{ear length}) + [12.239 \times (\text{tail length/body length})]$

$D > 0 = P. leucopus$

$D < 0 = P. maniculatus$

Percent probability of correct classification: 97.9%.

Adults: $D = -48.184 + (0.279 \times \text{body length}) + (-0.295 \times \text{tail length}) + (0.788 \times \text{hind foot length}) + [37.849 \times (\text{tail length/body length})]$

$D > 0 = P. leucopus$

$D < 0 = P. maniculatus$

(0.5 added to constant to standardize cut-off score to 0)

Percent probability of correct classification: 98.6%.

Skulls of adult and old adult deer mice and white-footed mice collected from southern Illinois can also be accurately identified using the following equations:

Adults: $D = -42.250 + (0.489 \times \text{zygomatic breadth posterior}) + (-0.642 \times \text{zygomatic breadth anterior}) + (0.876 \times \text{interorbital constriction}) + (0.951 \times \text{greatest rostral breadth}) + (1.078 \times \text{basinasal length}) + (2.198 \times \text{length of maxillary tooththrow}) + (1.542 \times \text{breadth across molars}) + (1.418 \times \text{cranial depth}) + (-1.501 \times \text{nasal length})$

$D > 0 = P. leucopus$

$D < 0 = P. maniculatus$

(0.5 added to constant to standardize cut-off score to 0)

Percent probability of correct classification: 98.9%.

Old Adults: $D = -62.831 + (1.094 \times \text{zygomatic breadth posterior}) + (0.528 \times \text{basinasal length}) + (2.370 \times \text{total length of tooththrow}) + (1.727 \times \text{cranial depth}) + (-0.693 \times \text{nasal length})$

$D > 0 = P. leucopus$

$D < 0 = P. maniculatus$

(0.5 added to constant to standardize cut-off score to 0)

Percent probability of correct classification: 100.0%.

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