Field Identification of the Mice *Peromyscus leucopus noveboracensis* and *P. maniculatus gracilis* in Central New York

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Field identification of the White-footed Mouse (*Peromyscus leucopus noveboracensis*) and Long-tailed Deer Mouse (*Peromyscus maniculatus gracilis*) is difficult because of their similar external morphology. *Peromyscus* were sampled by live-trapping during a five-year period (1992-1996) at the Arnot Teaching and Research Forest, Van Etten, New York and identified to species by electrophoresis of their salivary amylase. No electromorphs were shared between *P. leucopus* and *P. maniculatus*, thus permitting unambiguous species identification of individuals. Means and ranges of four external measurements (ear, head-body, hind-foot, and tail) and tail to head-body ratio were determined for amylase-genotyped live mice. Although some body measurements did differ on average between the two species (ear, head-body, and tail for adults; hind-foot and tail for juveniles), the ranges of these overlap considerably. When the four external measurements (excluding the tail to head-body ratio) were used to construct two discriminant-function equations, they yielded correct identification of 80% of the adult *P. l. noveboracensis* and *P. m. gracilis* assessed excluding juveniles, and 71% of adult and juvenile mice combined. The function reported here allows partial field identification, but genetic analysis remains the only reliable field method for differentiation between live *P. l. noveboracensis* and *P. m. gracilis*.

Key Words: *Peromyscus leucopus*, *Peromyscus maniculatus*, identification, salivary amylase, external characters, discriminant-function.

In the northeastern United States and southeastern Canada, the Deer Mouse, *Peromyscus maniculatus*, and the White-footed Mouse, *Peromyscus leucopus*, are sympatric over a large portion of their ranges (Klein 1960; Smith and Speller 1970; Grant 1976; Parren and Capen 1985; Garman et al. 1994; Long 1996). In regions where the White-footed Mouse, *P. l. noveboracensis*, and the long-tailed subspecies of the Deer Mouse, *P. m. gracilis*, are both present, field identification has proven to be difficult.

Characteristics of the tail and pelage have been cited as useful in differentiating the two species (Osgood 1909; Choate 1973; Whitaker and Hamilton 1998). Choate (1973) found that *P. l. noveboracensis* has a tail to head-body length ratio of < 1, whereas *P. m. gracilis* has a ratio that approaches or exceeds 1. Several researchers, however, have reported that external phenotypic characteristics in the field are unreliable (Grant 1976; Feldhamer et al. 1983; Palas et al. 1992; Garman et al. 1994; Rich et al. 1996; Sternburg and Feldhamer 1997; Kamler et al. 1998; Bruseo et al. 1999).

Discriminant-function equations using cranial measurements to distinguish between *P. l. noveboracensis* and *P. m. gracilis* have been constructed (Choate 1973; Long and Long 1993; Rich et al. 1996; Sternburg and Feldhamer 1997), but this method requires the sacrifice of individuals, and thus is not useful for on-going ecological studies. Stromberg (1979) developed a discriminant-function with field measurements for separating *P. l. noveboracensis* and *P. m. bairdii* in southern Wisconsin, but this function can not be used to discriminate between the two long-tailed subspecies. For identification of *P. l. noveboracensis* and *P. m. gracilis*, Garman et al. (1994) developed a discriminant-function equation with the tail-to-body length ratio as the independent variable, but they did not report the equation or its ability to discriminate between species. Feldhamer et al. (1983) and Sternburg and Feldhamer (1997) constructed discriminant-functions using only external characteristics of *P. l. noveboracensis* and *P. m. gracilis* and classified 98.6% of the *Peromyscus* correctly. Their measurements, however, were taken from dead individuals and thus are more accurate than is possible with live mice. Finally, Bruseo et al. (1999) used external field measurements in a discriminant analysis to differentiate *P. l. noveboracensis* and *P. m. nubiterrae* in the Appalachian Mountains. Although their models correctly classified up to 92% of live individuals, the authors concluded that electrophoresis of salivary amylase is the only technique that provides unambiguous identification of these two subspecies.

Peromyscus maniculatus and *P. leucopus* have unique salivary amylase variants and therefore can be accurately identified by their amylase allozyme genotype (Aquadro and Patton 1980). Aquadro and Patton's (1980) approach has been employed successfully in various ecological studies of eastern *Peromyscus* to improve the accuracy of field identifications (Feldhamer et al. 1983; Merriam et al. 1989; Palas et al. 1992; Garman et al. 1994; Rand et al. 1993; Kilpatrick et al. 1994; Rich et al. 1996; Sternburg and Feldhamer 1997; Bruseo et al. 1999). Several researchers have concluded that identification of *P. maniculatus* and *P. leucopus* in northeastern North America can be reliably done only using molecular markers (Feldhamer et al. 1983; Palas et al. 1992; Kilpatrick et al. 1994; Rich et al. 1996; Sternburg and Feldhamer 1997). Because identification with salivary amylase is time consuming and requires an extensive laboratory analysis, a method for quick field identification would be beneficial for mark-recapture studies.

The primary objective of this study was to improve field identification of *P. leucopus noveboracensis* and *P. maniculatus gracilis* for field studies and to extend the results of Bruseo et al. (1999) to another geographic location with a different subspecies of *P. maniculatus*. *Peromyscus* live-trapped in central New York State over a five-year period were identified to species through electrophoretic analysis of their salivary amylase. We also examined the usefulness of external and measured characters cited previously for the identification of these two sympatric species. We then statistically tested the trend cited by Choate (1973) for tail to head-body length ratio of each species. In addition, two discriminant classification equations were derived from four external body measurements of all individuals discerned to species by the electrophoretic analysis.

Methods

Field Methods

Peromyscus leucopus noveboracensis and *P. maniculatus gracilis* were live-trapped on three study plots in the Arnot Teaching and Research Forest of Cornell University, Van Etten, New York, USA (42° 17' 30" N, 76° 40' 00" W; altitude= 500-550 m). The two species are known to dwell in forests in the northeastern United States, northern Michigan, Wisconsin, and southeastern Canada, and are often sympatric (Hamilton 1943; Baker 1983; Whitaker and Hamilton 1998). The Prairie Deer Mouse, *P. m. bairdii*, also has been trapped previously in Tompkins Co., New York (the northeasternmost part of the subspecies' range) in open field habitats (Whitaker and Hamilton 1998). From our tail measurements of trapped *P. maniculatus*, we are certain all our specimens belong to the *P. m. gracilis* subspecies.

Two of the trapping plots were in un-mowed pasture (old field habitat), and the other was located in a transition forest that was logged in the early 1900s and dominated by red maple (*Acer rubrum*) and beech (*Fagus grandifolia*). One hundred Sherman live traps were spaced 5 m apart in 10 by 10 grids in each plot. As part of a mark-recapture study, the plots were trapped from late May to early November of 1992-96 for two nights each week. Traps were open between 1800 and 0600 hrs with two trap checks (1200 and 0600 hrs) each night.

All mammals captured alive were identified to genus, weighed, measured, and sexed. We took quantitative body measures of each *Peromyscus* at first capture only. We recorded tail length, head-body length, ear length, and hind-foot length to the nearest mm using a clear, flexible plastic ruler. At first capture we collected a saliva sample (Aquadro and Patton 1980). Field assistants did not attempt to identify *Peromyscus* to species in the field.

Laboratory Methods

We genotyped saliva samples for salivary amylase using Aquadro and Patton's (1980) electrophoretic method. A known sample from the *Peromyscus* Stock Center with the Amy-1^{100} electromorph of *P. leucopus* was added to each gel as a standard (Dawson and Ward 1994); amylase electromorphs were then designated by percentage mobility relative to Amy-1^{100} mobility. The Amy-1^{100} electromorph is only found in *P. leucopus* (Aquadro and Patton 1980).

Statistical Analyses

We genotyped only those samples with distinct bands. Once the species of each individual was determined electrophoretically, differences between the body measurements of each species could be reliably assessed. All 220 *P. maniculatus* and *P. leucopus* identified to species from the five years and three trapping plots were grouped together in their respective species and age groups for our statistical analyses. We chose weight as the determining factor for age because it reflects the relative size of the individual. We classified an individual as an adult if it weighed $\geq$ 15 g, and as a juvenile if it weighed < 15 g (Wolff 1985). We used two-tailed Student's *t*-tests to compare the adult and juvenile lengths of the tail, head-body, hind-foot, ear, and tail to head-body ratio between each species (MINITAB Inc. 1995). In addition, within each species we compared the tail and head-body lengths of each individual mouse with a paired *t*-test.

We developed two discriminant-function equations: one for adults, and one for adults and juveniles combined (SYSTAT Inc. 1992). We used four variables: tail length, head-body length, hind-foot length, and ear length. The discriminant analysis optimally weights the four body measurement variables to segregate the two species. The discriminant-functions can be used to predict the species of new, unclassified individuals.

Results

We collected 270 salivary amylase samples from individuals trapped in the three plots over five years (1992-1996). Of these, 220 samples had sufficient

amylase for electrophoresis. In 55 of the samples analyzed, we found enzymatic degradation that made distinguishing between homozygotes and heterozygotes at the amylase locus difficult (probably due to thawing and refreezing during transportation and storage). In these cases species identification was unambiguous, but exact genotype was not scored. Thus, we identified 220 individuals to species, and scored the genotypes of 165 individual *Peromyscus*.

We found two salivary amylase electromorphs, Amy-1^{76} and Amy-1^{85}, in frequencies of 81.7% and 18.3% (N= 186) respectively that were unique to *P. maniculatus*. *P. leucopus* carried two different alleles, Amy-1^{94} and Amy-1^{100}, which we found at frequencies of 88.2% and 11.8% (N= 144).

P. maniculatus gracilis was significantly larger than *P. leucopus noveboracensis* in head-body, tail, and ear lengths for adults and juveniles (Table 1). Tail lengths for adults and juveniles of *P. maniculatus* were significantly longer than those for *P. leucopus* (t = -9.47, d.f. = 148, $P < 0.0001$; and t = -3.02, d.f. = 67, $P = 0.004$ respectively). The head-body lengths for adult *P. maniculatus* were significantly longer than that of *P. leucopus* (t = -4.98, d.f. = 149, $P < 0.001$), but the juvenile head-body lengths between species were not (t = -1.33, d.f. = 67, $P = 0.19$). The ear lengths of adult *P. maniculatus* also were significantly longer than those of adult *P. leucopus* (t = -3.58, d.f. = 145, $P < 0.001$), whereas the ear lengths of juvenile *P. maniculatus* were not longer than those of *P. leucopus* (t = -1.44, d.f. = 67, $P = 0.16$). Adult hind-foot lengths did not differ between species (t = -0.586, d.f. = 149, $P = 0.56$), but juvenile *P. maniculatus* had longer hind-feet than *P. leucopus* (t = -1.08, d.f. = 67, $P < 0.001$).

For both the adult and juvenile groups of *P. m. gracilis* and *P. l. noveboracensis*, tail length of an individual mouse was found to be greater than head-body length (paired t-test; = -10.4, d.f. = 98, $P < 0.001$ for *P. maniculatus* adults; t = -3.20, d.f. = 50, $P = 0.0024$ for *P. leucopus* adults; t = -7.08, d.f. = 51, $P < 0.0001$ for *P. maniculatus* juveniles; and t = -3.43, d.f. = 16, $P = 0.0034$ for *P. leucopus* juveniles). The individual tail to head-body ratio on *P. maniculatus* was significantly greater than the ratio for *P. leucopus* in adults, but not for juveniles (t = -2.44, d.f. = 149, $P < 0.016$ for adults; and t = -1.56, d.f. = 67, $P = 0.12$ for juveniles). Both ratios were on average equal to or greater than 1 (Table 1).

The first pair of discriminant-function equations (Table 2a), constructed for adults, classified 80.1% (117 of 146 total) of the *Peromyscus* into their correct species groups (Figure 1a). Of 97 *P. maniculatus* tested, 21 (21.6%) were misidentified, and of 49 *P. leucopus*, 8 (16.3%) were not classified correctly.

The addition of juveniles in the discriminant-function lowered its success rate. The second pair of equations (Table 2b) constructed with adults and juveniles, classified 71.6% (154 of 215 total) of the *Peromyscus*

TABLE 1. Measurements of four morphological measurement variables and the tail to head-body ratio for *P. l. noveboracensis* and *P. m. gracilis*, near Van Etten, New York in 1992-1996. Significance values refer to interspecies, within age comparisons by t-tests and are noted at larger mean value.

Length (mm)	*P. maniculatus*						*P. leucopus*					
	Juvenile			Adult			Juvenile			Adult		
	$\bar{X}$	SD	range	$\bar{X}$	SD	range	$\bar{X}$	SD	range	$\bar{X}$	SD	range
Ear	14.6	2.7	7-23	15.3***	2.3	10-21	13.6	2.4	9-17	13.9	2.0	9-17
Hind-Foot	18.7***	1.6	11-21	19.2	1.4	16-22	18.2	1.7	15-20	19.0	1.5	15-23
Head-Body	67.3	6.4	53-80	74.1***	6.9	58-94	64.9	6.4	52-75	68.5	5.8	54-86
Tail	76.5**	7.3	51-88	84.1***	7.2	70-105	70.3	7.9	57-90	72.2	7.4	50-88
Tail/ Head-Body	1.15	0.15	0.77-1.47	1.13***	0.14	0.88-1.55	1.09	0.11	0.88-1.38	1.06	0.13	0.82-1.49
Sample size (n)[a, b]	52			100			17			51		

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

[a] Ear length values are for 98 and 49 adult *P. maniculatus* and *P. leucopus* respectively because of missing data.

[b] *P. maniculatus* tail length values are for 99 mice only.

TABLE 2. Group classification coefficients for discrimination between *P. l. noveboracensis* and *P. m. gracilis* using external characteristics from N=220 individuals caught near Van Etten, New York in 1992-1996.

	a. Adults only		b. Adults and Juveniles	
Variable	*P. leucopus* n= 49	*P. maniculatus* n= 97	*P. leucopus* n= 66	*P. maniculatus* n= 149
Ear	1.205	1.331	0.552	0.668
Head-body	1.301	1.405	0.812	0.849
Hind-foot	9.642	9.724	7.263	7.231
Tail	1.388	1.608	0.889	1.033
Constant	-195.810	-223.871	-132.300	-146.965

correctly (Figure 1b). Of 149 *P. maniculatus* tested, 44 (29.5%) were misidentified, whereas of 66 *P. leucopus*, 17 (25.8%) were not classified correctly.

Discussion

We were able to identify unambiguously individuals captured as *P. leucopus noveboracensis* or *P. maniculatus gracilis* using salivary amylase genotype. No electromorphs were shared between the two species. We found both species to be polymorphic at the amylase locus. The alleles found in our study (Amy-1^{76} and Amy-1^{85} for *P. maniculatus*, and Amy-1^{94} and Amy-1^{100} for *P. leucopus*) were consistent with those found in previous studies (Aquadro and Patton 1980; Feldhamer et al. 1983; Merriam et al. 1989; Palas et al. 1992; Kilpatrick et al. 1994; Rich et al. 1996; Sternburg and Feldhamer 1997; Bruseo et al. 1999). Aquadro and Patton (1980) reported only the Amy-1^{76} and the Amy-1^{100} in their New York *P. maniculatus* and *P. leucopus*, respectively, but had assayed only four individuals of each species. Our larger sample size allowed us to detect the less frequent alleles in *P. leucopus* and *P. maniculatus.*

Our ranges for lengths of tail for *P. l. noveboracensis* and *P. m. gracilis* were similar to previously reported values (Godin 1977; Baker 1983; Whitaker and Hamilton 1998). We found that hind-foot length was not a reliable distinguishing characteristic for the two species as also suggested by others (Baker 1983; Feldhamer et al. 1983; Palas et al. 1992; Whitaker and Hamilton 1998). Ear lengths were more variable than other reported ranges, and have resulted from the difficulties measuring live mice in the field (Baker 1983; Feldhamer et al. 1983; Sternburg and Feldhamer 1997).

Although *P. m. gracilis* were on average larger than *P. l. noveboracensis*, their body measurements overlapped considerably making identification difficult (Table 1). For example, tail lengths of adult *P. leucopus* range from 50-88 mm, whereas tail lengths of adult *P. maniculatus* range from 70-105 mm. In the range of 70-88 mm, a mouse could be classified as a member of either species if tail length was used as the sole identification characteristic. Furthermore, the area of overlap in body measurements is greater for ear, hind-foot, and head-body lengths than for tail length. Tail to head-body ratio values of the two species also overlapped considerably (Table 1). If body dimensions were solely used to identify *Peromyscus*, ambiguous identifications would thus be obtained.

FIGURE 1A

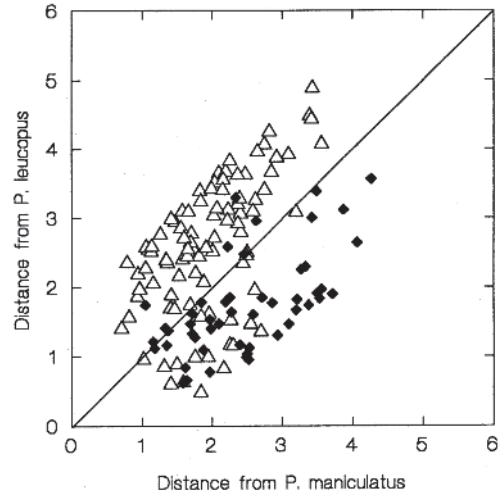

FIGURE 1B

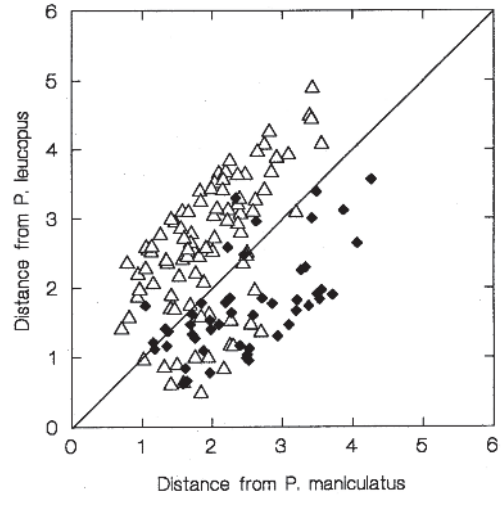

FIGURE 1. Classification of *P. l. noveboracensis* and *P. m. gracilis* using the discriminant-function coefficients given in Table 2. Figure 1a shows the classification of adults only. Figure 1b shows the classification of adults and juveniles. Open and closed symbols denote *P. m. gracilis* and *P. l. noveboracensis*, respectively. In Figure 1b, *P. m. gracilis* is indicated by open circles and squares (adult and juvenile, respectively), and *P. l. noveboracensis* by closed triangles and stars (adult and juvenile, respectively).

Choate (1973) reported that the tail to head-body ratio was valuable for species identification of *P. l. noveboracensis* and *P. m. gracilis*, with *P. l. noveboracensis* having a ratio < 1. Although the ratio was larger for *P. m. gracilis* than *P. l. noveboracensis* in our study, the two species had ratios ≥ 1 (Table 1). In previous studies where head-body lengths were taken from dead specimens, the average tail to head-body ratios for both species were < 1, although *P. l. noveboracensis* had larger ratios than either *P. m. bairdii* or *P. m. nubiterrae* (Feldhamer et al. 1983; Sternburg and Feldhamer 1997). We found that the tail to head-body ratio is not a diagnostic characteristic for the field identification of *P. l. noveboracensis* and *P. m. gracilis* in central New York as did Bruseo et al. (1999).

Juvenile *Peromyscus* demonstrated the same trends observed in adult *Peromyscus* for tail, and tail to head-body length ratio. When juveniles were included with adults in the second discriminant-function equation, the success rate of the classification function decreased (Figure 1b). Some researchers have been concerned with including juveniles in their discriminant-functions or standard *t*-tests. Palas et al. (1992) reported that misidentification using body measurements is most probable in the young age classes. Choate (1973) excluded juveniles when constructing his discriminant-function. On the other hand, Rich et al. (1996) included juveniles in their function even though significant differences were found between age classes within species. We agree with Rich et al.'s (1996) reasoning that the purpose of a discriminant-function analysis is to construct a function that classifies all specimens to species independent of age or size.

The discriminant-function reported here will enable researchers to partially differentiate between the two species in a live-trapping study. Yet we also have confirmed the unreliability of identifications that depend solely on external characteristics. While our discriminant-functions are an improvement from the independent external measurements and tail to head-body ratio, they still have a degree of ambiguity. Unambiguous identification of live *P. l. noveboracensis* and *P. m. gracilis* in the field still appears to be only possible by genetic analysis.

Acknowledgments

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Erratum Canadian Field-Naturalist 117(1)

Ballard, Warren B., Matthew A. Cronin, Martin D. Robards, and **William A. Stubblefield.** 2003. Heavy metal concentrations in Arctic Foxes, *Alopex lagopus,* in the Prudhoe Oil Field, Alaska. Canadian Field-Naturalist 117(2): 119-121.

The abbreviation for concentrations in micrograms per gram of dry weight should be corrected in two places. On page 120 "mg/g" left column line 18, and right column line 21, should be "µg/g".

Erratum Canadian Field-Naturalist 117(2)

Lindquist, E. S., C. F. Aquadro, D. McClearn, and **K J. McGowan.** 2003. Field identification of the mice *Peromyscus leucopus noveboracensis* and *P. maniculatus gracilis* in central New York. Canadian Field-Naturalist 117(2): 184-189.

On page 4, Figure 1, 1A was repeated for 1B. The correct 1B is shown below with 1A.

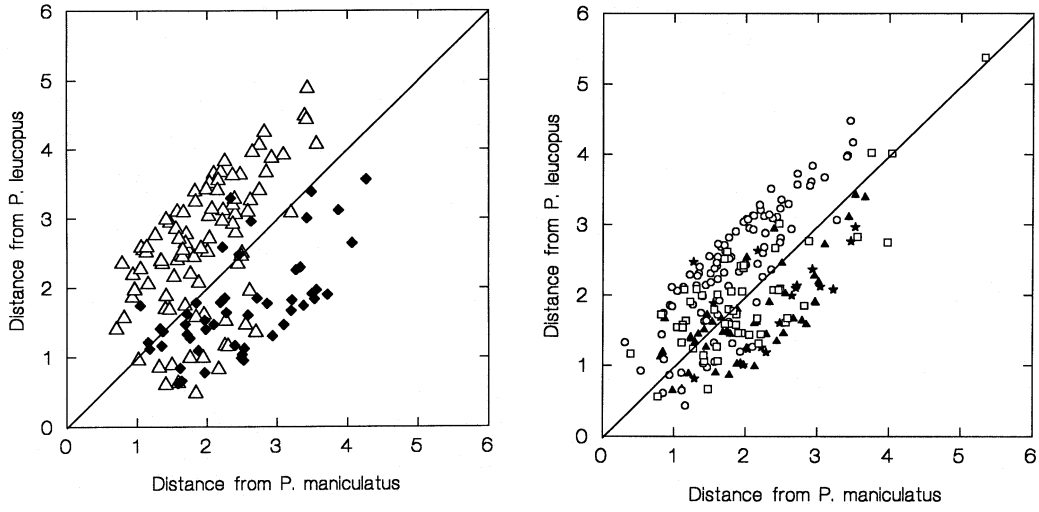

FIGURE 1. Classification of *P. l. noveboracensis* and *P. m. gracilis* using the discriminant-function coefficients given in Table 2. Figure 1a shows the classification of adults only. Figure 1b shows the classification of adults and juveniles. Open and closed symbols denote *P. m. gracilis* and *P. l. noveboracensis*, respectively. In Figure 1b, *P. m. gracilis* is indicated by open circles and squares (adult and juvenile, respectively), and *P. l. noveboracensis* by closed triangles and stars (adult and juvenile, respectively).