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Field Discrimination of Prairie Deer Mice and White-footed Mice using Morphological Characteristics

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ABSTRACT Field discrimination of prairie deer mice (*Peromyscus maniculatus bairdii*) and white-footed mice (*P. leucopus noveboracensis*) can be difficult throughout much of the central United States where they co-occur. We live-trapped prairie deer mice and white-footed mice within forested and row-crop habitats in central Indiana and used multiplex PCR with species-specific primers to positively determine species identification. We collected a suite of commonly measured external morphological traits (body weight and lengths of ear, hind foot, tail, and body) from each captured animal. Individuals were assigned to species based on analysis of DNA; discriminant function analysis was used to identify morphological characteristics that best distinguished the two species. Tail length was the best single discriminator (95.4% discrimination efficiency), with prairie deer mice having shorter tails than white-footed mice. When tail length was used in conjunction with hind foot length, we were able to correctly discriminate 96.8% of individuals in our sample. Our results provide simple metrics for field identification of prairie deer mice and white-footed mice in the prairie peninsula region of central Indiana.

KEY WORDS discriminant analysis, identification, morphology, *Peromyscus leucopus noveboracensis*, *Peromyscus maniculatus bairdii*, sympatry.

Prairie deer mice (*Peromyscus maniculatus bairdii*) and white-footed mice (*Peromyscus leucopus noveboracensis*) are common and widely distributed rodents in the central United States and have considerable range overlap throughout much of this region. The two species are widely sympatric at large spatial scales; however, they are conventionally considered to occur allotopically (sensu Rivas 1964) because prairie deer mice select open or sparsely vegetated habitats, whereas white-footed mice select forested habitat and areas with dense vegetation structure (Kaufman and Fleharty 1974, M'Closkey 1975). Nonetheless, both species are considered generalists (Swihart et al. 2003, Swihart et al. 2006) and show flexibility in habitat use such that populations can exhibit syntopy in certain transitional habitats such as row-crops, prairie edges, and old fields in fragmented agroecosystems (e.g., Clark and Young 1987, Kamler et al. 1998). Prairie deer mice and white-footed mice are morphologically similar and difficult to distinguish when in syntopy (Kamler et al. 1998), making species-specific assessments of habitat use and population dynamics challenging. Furthermore, deer mice and white-footed mice are the primary reservoirs for Hantavirus (Mills et al. 1999) and Lyme disease (Donahue et al. 1987), respectively; hence, accurate species identification is important for disease surveillance purposes.

Several authors have provided discrimination criteria for deer mice and white-footed mice throughout their broad ranges. However, the majority of these assessments have distinguished woodland subspecies of deer mice (i.e., *P. maniculatus gracilis* and *P. maniculatus nubiterrae*) from white-footed mice where the ranges of these subspecies overlap throughout forested habitats of the eastern and

northern United States (Feldhamer et al. 1983, Long and Long 1993, Rich et al. 1996, Bruseo et al. 1999, Stephens et al. 2014). Woodland subspecies of deer mice typically have longer tails and ears than white-footed mice, and these external characteristics are useful in species identification (Feldhamer et al. 1983, Rich et al. 1996, Lindquist et al. 2003, Stephens et al. 2014). Conversely, prairie deer mice are typically smaller-bodied and have shorter appendages than white-footed mice (Choate et al. 1979, Sternburg and Feldhamer 1997), likely because of adaptations to grassland environments; therefore, characteristics that discriminate woodland subspecies of deer mouse from white-footed mice are not necessarily applicable to the prairie subspecies. Few studies have provided morphological criteria to discriminate prairie deer mice and white-footed mice, and only one (Sternburg and Feldhamer 1997) has used salivary amylase or genetic markers to confirm species identity of reference specimens. Sternburg and Feldhamer (1997) found that both external (tail-body length ratio) and cranial measurements were useful in distinguishing the two species in southern Illinois. However, due to high intraspecific morphological variation among *Peromyscus* populations, regionally specific discrimination criteria are necessary for accurate identification of prairie deer mice and white-footed mice throughout areas where ranges overlap. Therefore, our objectives were to 1) use genetic markers to positively identify prairie deer mice and white-footed mice captured within forest and row-crop habitats in the prairie peninsula region of west-central Indiana, and 2) use discriminant analysis to evaluate and identify external measurements that accurately distinguish prairie deer mice from white-footed mice in the field.

Table 1. Descriptive statistics (mean, range, and standard error [SE]) of external measurements from 54 prairie deer mice (*Peromyscus maniculatus bairdii*) and 100 white-footed mice (*Peromyscus leucopus noveboracensis*) sampled in forest ($n = 72$) and row-crop field ($n = 28$) habitats in west-central Indiana between May – August, 2015.

	<i>P. m. bairdii</i>			<i>P. l. noveboracensis</i> (forest)			<i>P. l. noveboracensis</i> (field)		
	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range
Mass (g)	16.1	0.6	7 – 28	18.8	0.6	8 – 31	19.6	0.9	12 – 29
Tail length (mm)	53.5	0.7	38 – 65	73.2	0.7	52 – 89	73.4	1.0	64 – 83
Hind foot length (mm)	17.1	0.2	12 – 21	20.1	0.2	16 – 23	20.6	0.2	18 – 23
Ear length (mm)	12.8	0.2	10 – 17	14.6	0.2	11 – 20	14.7	0.3	12 – 17
Body length (mm)	74.4	1.1	60 – 90	76.6	0.7	57 – 95	77.1	1.4	61 – 90

METHODS

Field Methods

We collected ear tissue and morphological measurements from prairie deer mice and white-footed mice while live-trapping as part of a concurrent investigation of small mammal use of habitat edges in fragmented agro-ecosystems. We used rectangular 2.52-ha grids of Sherman live traps ($7.62 \times 8.89 \times 22.86$ cm) with 20-m spacing (10×8) to capture prairie deer mice and white-footed mice from 8 woodlots and adjoining row-crop (corn and soybean) fields in Tippecanoe County, Indiana, from 10 May – 7 August 2015. Trapping grids straddled forest-field edges, such that both forest and row-crop habitats were simultaneously sampled. Upon initial capture, we uniquely marked individuals > 7 g with passive integrated transponder (PIT) tags (Biomark, Boise, ID) and collected a small ear tissue sample for genetic species identification (see laboratory methods below); samples were kept frozen until laboratory processing. We collected external measurements from each individual following Stephens et al. (2014), which included: ear length (basal notch to tip, excluding hairs), hind foot length (calcaneus to longest claw), tail length (sacrum to caudal tip, excluding hairs), body length (tip of nose to basal tail), and weight. All external measurements were estimated to the nearest mm using a flexible plastic ruler, and we measured weight to nearest gram using a Pesola® scale. Field technicians were trained with laboratory specimens prior to collecting in-field measurements to reduce the likelihood of observer error.

Laboratory Methods

We identified captured mice to species using the genetic approach described by Tessier et al. (2004). We extracted DNA from ear tissue samples using Qiagen DNeasy blood and tissue kits (Qiagen, Mississauga, Ontario) following manufacturer-recommended protocols. We then ran multiplex polymerase chain reactions (PCR) with species-specific primers of different length (225 and 159 base pair fragments

for deer mice and white-footed mice, respectively; Tessier et al. 2004). We evaluated amplification success by running PCR products on 3% agarose stained with ethidium bromide, and we determined species membership (deer mouse or white-footed mouse) by number of base pairs amplified and measured against an in-house developed 100 base pair ladder standard.

Data Analyses

We calculated descriptive statistics (mean, range, and standard error) of external measurements for prairie deer mice and white-footed mice and used multivariate tests (Hotelling's T^2 and Levene's F) to assess differences in mean vectors and homogeneity of variance-covariance structure between species, sexes, and for individuals captured in different habitat types (i.e., forest and row-crops). We used quadratic discriminant analysis (QDA) and linear discriminant analysis (LDA) to identify morphological characteristics that best discriminated prairie deer mice and white-footed mice. Because both methods yielded similar conclusions, we present results from the LDA analysis for ease of interpretation and implementation. We developed discriminant functions with all possible 3-variable combinations of externally measured variables. We then used 10-fold cross-validation of each function to evaluate discrimination efficiency in species identification. All analyses were conducted in Program R (R Core Development Team 2016).

RESULTS

We captured and collected external measurements from 154 individual *Peromyscus*. Subsequent genetic analysis identified 54 prairie deer mice and 100 white-footed mice. White-footed mice were captured in both forest ($n = 72$) and row-crop ($n = 28$) habitat, whereas prairie deer mice were only captured in row-crop fields. There were no differences in either the mean vector of external characteristics between white-footed mice captured in forest and row-crop habitats ($T_{25,94} = 0.45$, $P = 0.81$; Table 1) or between sexes for either

Table 2. Standardized coefficients from discriminant function analyses of external measurements from prairie deer mice (*Peromyscus maniculatus bairdii*) and white-footed mice (*Peromyscus leucopus noveboracensis*) sampled in forest and row-crop habitats in west-central Indiana between May – August, 2015. Discrimination efficiencies were calculated from 10-fold cross-validation.

Standardized Coefficients						
Function	Tail length (mm)	Hind foot length (mm)	Ear length (mm)	Body length (mm)	Total Wt (g)	Discrimination Efficiency (%)
1	-0.143	-0.119				96.8
2	-0.163	-0.113		0.055		96.8
3	-0.177				-0.071	96.1
4	-0.163	-0.119			0.071	96.1
5	-0.169		-0.177		0.085	96.1
6	-0.141	-0.101	-0.065			96.1
7	0.158					95.5
8	-0.152		-0.102			94.8
9	-0.178			-0.055		94.7
10	0.182			0.042	0.035	94.7
11	0.168		-0.195	-0.067		94.7
12		-0.466	-0.239			85.7
13		-0.466	-0.239		-0.001	85.7
14		-0.466	-0.271	0.017		84.4
15		-0.555		0.014	-0.035	84.4
16		0.571				83.8
17		-0.570		-0.001		83.8
18		-0.553			-0.022	83.8
19			-0.711	-0.019		77.9
20			-0.651		-0.018	77.9
21			-0.671	0.039	-0.051	77.9
22			0.678			74.7
23					0.192	66.2
24				0.013	-0.204	66.2
25				0.132		64.9

species ($T^2_{5,99} = 0.69$, $P = 0.62$ for *P. leucopus* and $T^2_{5,48} = 0.67$, $P = 0.64$ for *P. maniculatus*). Moreover, no differences existed in variance-covariance structure for white-footed mice in the two habitats ($F_{1,98} = 0.04$, $P = 0.82$) or between sexes of either species ($F_{1,98} = 1.15$, $P = 0.28$ for *P. leucopus* and $F_{1,52} = 0.06$, $P = 0.79$ for *P. maniculatus*). We therefore pooled individuals from both sexes and habitat types for further analysis. Collectively, mean external characteristics differed significantly between species ($T^2_{5,148} = 99.01$, $P < 0.001$). On average, prairie deer mice had shorter tails, hind feet, and ears than white-footed mice (Table 1).

Discriminant models that included tail length and hind

foot length achieved the highest discrimination efficiency (Table 2). The function including only tail length provided excellent cross-validated discrimination efficiency, correctly classifying species 95.4% of the time. The function for hind foot length also achieved relatively high discrimination efficiency (83.8%). When hind foot length was included with tail length, cross-validated discrimination efficiency increased slightly, to 96.8%. This bivariate function misclassified 5 individuals, of which 4 were white-footed mice (3 from forest and 1 from field; Fig. 1). Functions for weight or ear and body lengths generally had poor discrimination efficiency (i.e., < 80%) unless they were combined with tail length or hind

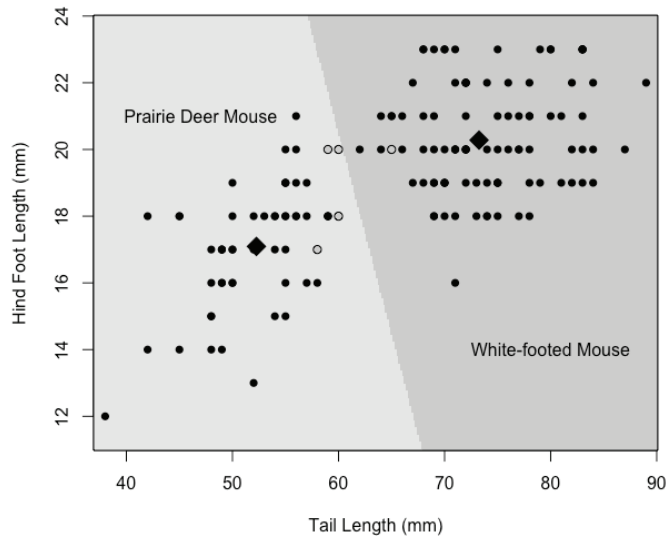


Figure 1. Predicted identification of prairie deer mice and white-footed mice based on discriminant function analysis of hind foot length and tail length. Solid black circles represent individual mice correctly classified by discriminant model, whereas unfilled circles represent misclassified individuals ($n = 5$; misclassification rate = 3.2%). Diamonds represent mean values for both species.

foot length. The LDA classification equations for the top-performing function (tail length + hind foot length) were: *P. leucopus* = $-98.9 + 1.36 \times \text{tail length} + 4.84 \times \text{hind foot length}$ and *P. maniculatus* = $-60.7 + 0.87 \times \text{tail length} + 4.44 \times \text{hind foot length}$.

DISCUSSION

Due to intraspecific variation and overlap in morphological characteristics between species, none of the external measurements evaluated in this study provided species identification without error. Previous authors have noted similar patterns for prairie deer mice and white-footed mice in southern Illinois (Sternburg and Feldhamer 1997) and eastern Kansas (Choate et al. 1979) where no single morphological characteristic provided unambiguous species identification. Despite slight morphological overlap between species in our study, tail length and hind foot length can be used for efficient species identification in west-central Indiana.

Previous work by Choate et al. (1979) and Sternburg and Feldhamer (1997) in eastern Kansas and southern Illinois, respectively, indicated that both skull morphology and external body measurements are useful in discriminating *Peromyscus*. Choate et al. (1979) found skull morphology to best discriminate prairie deer mice and white-footed mice and concluded that external characteristics were generally unreliable in species identification. Conversely, Sternburg and Feldhamer (1997) found that among externally measured

characteristics, the ratio of tail length and body length best discriminated the two species. Although we did not measure skull morphology, our external body measurements (means and ranges) differed slightly from these previous studies, perhaps due to regional morphological variation in *Peromyscus*; our mean morphological measurements differed by $> 15\%$ in some cases. Alternatively, it is possible that differences were due to sampling from populations with markedly different survival and recruitment and thus different age and size structures, potential sources of variation that we do not consider here, but that warrant further study. Regardless of the cause, such high among-population variation further highlights the need for regionally appropriate discriminant functions for species identification.

Regional variation in morphology is well documented for both deer mice and white-footed mice (e.g., Choate et al. 1979). In fact, intraspecific variation in morphology of *Peromyscus* species can be so extreme that previous authors have documented variation among individuals occupying different adjoining habitat types in the same general area. For example, Kamler et al. (1998) found that white-footed mice captured in old field habitat in Kansas differed morphologically from their conspecifics in adjoining forested habitat and instead resembled prairie deer mice, which were common in old fields. However, their sample was limited to two individual white-footed mice collected from old fields for their comparative analysis. We implemented an analogous experimental design and sampled individual white-footed mice from both allotopic (forest, $n = 72$) and syntopic (row-crop, $n = 28$) habitats, but we found no significant difference in morphology between mice in these habitats. Consequently, there does not appear to be a discernible morphological difference in white-footed mice when occupying habitats with or without prairie deer mice. Live-trapping before, during, and after the growing season in our study system has shown that white-footed mice do not occur in row-crops year-round; instead, they are seasonally resident only during summer crop growth (Abercrombie et al. 2017, Berl et al. 2017). Ephemeral use of row-crop habitat may explain a lack of morphological separation among white-footed mouse subpopulations, because regular genetic exchange among individuals from woodlot and field habitats would be possible annually during spring, fall, and winter.

Measurement error presents a challenge in evaluating field-obtained external morphological measurements from live-trapped mice (Blackwell et al. 2006, Stephens et al. 2015). However, in many cases sacrificing individual animals for species identification based on skull or cranial measurements is undesirable, and field identification with external measurements is the only feasible option. The two external characteristics that we found useful in species identification (tail length and hind foot length) are arguably two of the most straightforward and least subjective measurements typically collected from mice by field biologists (Bruseo et al. 1999).

However, all external body measurements collected from live animals can be prone to measurement error, particularly when multiple researchers participate in data collection (Blackwell et al. 2006). This is true more for hind foot length than tail length (Stephens et al. 2015). Although our field identification criteria should be easily transferable to and applied by other field biologists, standardization of measurement criteria, training of personnel, and replicate measurements on individuals are encouraged to reduce the likelihood of unreliable species identifications (Blackwell et al. 2006).

Accurate species identification is critical to species-specific investigations of habitat use, demography, and disease surveillance of sympatric rodents. Genetic or salivary amylase testing for species identification cannot be performed in the field and is often cost-prohibitive when large numbers of animals are captured (Stephens et al. 2014). Therefore, regionally appropriate field discrimination criteria based on external characteristics provide a rapid and cost-effective method of species identification. Our study provides field-based criteria that can be used to reliably discriminate sympatric prairie deer mice and white-footed mice in the prairie peninsula region of central Indiana.

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LITERATURE CITED

- Abercrombie, S. A., J. L. Berl, E. A. Flaherty, and R. K. Swihart. 2017. Seasonal foraging by forest mice enhances loss of weed seeds from crop field edges. *Northeastern Naturalist: In Press*.
- Berl, J. L., H. A. Johnstone, J. Y. Wu, E. A. Flaherty, and R. K. Swihart. 2017. Winter preference for weed seed and waste grain by native mice in row-crop agriculture. *Weed Science* 65:406–412.
- Blackwell, G. L., S. M. Bassett, and C. R. Dickman. 2006. Measurement error associated with external measurements commonly used in small-mammal studies. *Journal of Mammalogy* 87:216–223.
- Bruseo, J. A., S. H. Vessey, and J. S. Graham. 1999. Discrimination between *Peromyscus leucopus noveboracensis* and *Peromyscus maniculatus nutiberrae* in the field. *Acta Theriologica* 44:151–160.
- Choate, J. R. 1973. Identification and recent distribution of white-footed mice in New England. *Journal of Mammalogy* 54:41–50.
- Choate, J. R., R. C. Dowler, and J. E. Krause. 1979. Mensural discrimination between *Peromyscus leucopus* and *P. maniculatus* in Kansas. *Southwestern Naturalist* 24:249–258.
- Clark, W. R., and R. E. Young. 1987. Crop damage by small mammals in no-till cornfields. *Journal of Soil and Water Conservation* 41:338–341.
- Donahue, J. G., J. Piesman, and A. Spielman. 1987. Reservoir competence of white-footed mice for Lyme disease spirochetes. *American Journal of Tropical Medicine and Hygiene* 36:92–96.
- Feldhamer, G. A., J. E. Gates, and J. H. Howard. 1983. Field identification of *Peromyscus maniculatus* and *P. leucopus* in Maryland: reliability of morphological characteristics. *Acta Theologica* 28:417–423.
- Kamler, J. F., D. S. Pennock, C. Welch, and R. J. Pierotti. 1998. Variation in morphological characteristics of the white-footed mouse (*Peromyscus leucopus*) and the deer mouse (*P. maniculatus*) under allotopic and syntopic conditions. *American Midland Naturalist* 140:170–179.
- Kaufman, D. W., and E. D. Fleharty. 1974. Habitat selection by nine species of rodents in north-central Kansas. *Southwestern Naturalist* 18:443–452.
- 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* 117:184–189.
- Long, C. A., and J. E. Long. 1993. Discriminant analysis of geographical variation in long-tailed deer mice from northern Wisconsin and upper Michigan. *Transactions Wisconsin Academy of Science, Arts, Letters* 81:107–122.
- M'Closkey, R. T. 1975. Habitat dimensions of white-footed mice, *Peromyscus leucopus*. *American Midland Naturalist* 93:158–167.
- Mills, J. N., T. G. Ksiazek, C. J. Peters, and J. E. Childs. 1999. Long-term studies of hantavirus reservoir populations in the southwestern United States: a synthesis. *Emerging Infectious Diseases* 5:135–142.
- R Development Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Revis, L. R. 1964. A reinterpretation of the concepts “sympatric” and “allopatric” with proposal of the

- additional terms “syntopic” and “allotopic”. *Systematic Zoology* 13:42–43.
- Rich, S. M., C. W. Kilpatrick, J. L. Shippee, and K. L. Crowell. 1996. Morphological differentiation and identification of *Peromyscus leucopus* and *P. maniculatus* in northeastern North America. *Journal of Mammalogy* 77:985–991.
- Stephens, R. B., E. M. Anderson, S. R. Wendt and J. K. Meece. 2014. Field identification of sympatric *Peromyscus leucopus noveboracensis* and *P. maniculatus gracilis* in Wisconsin from external measurements. *American Midland Naturalist* 171:139–146.
- Stephens, R. B., K. H. Karau, C. J. Yahnke, S. R. Wendt, and R. J. Rowe. 2015. Dead mice can grow – variation of standard external mammal measurements from live and three postmortem body states. *Journal of Mammalogy* 96:185–193.
- Sternburg, J. E., and G. A. Feldhamer. 1997. Mensural discrimination between sympatric *Peromyscus leucopus* and *P. maniculatus* in southern Illinois. *Acta Theriologica* 42:1–13.
- Swihart, R. K., T. M. Gehring, M. B. Kolozsvary, and T. E. Nupp. 2003. Responses of ‘resistant’ vertebrates to habitat loss and fragmentation: the importance of niche breadth and range boundaries. *Diversity and Distributions* 9:1–18.
- Swihart, R. K., J. J. Lusk, J. E. Duchamp, C. E. Rizkalla, and J. E. Moore. 2006. The roles of landscape context, niche breadth, and range boundaries in predicting species responses to habitat alteration. *Diversity and Distributions* 12:277–287.

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