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Valerie A. Barko
Southern Illinois University Carbondale

Brian L. Sloss
Southern Illinois University Carbondale

George A. Feldhamer
Southern Illinois University Carbondale

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A Non-lethal Method for Identification of the Cotton Mouse, *Peromyscus gossypinus* (LeConte, 1853)

Valerie A. Barko[1], Brian L. Sloss[2], and G.A. Feldhamer[1]
[1]Department of Zoology, Southern Illinois University
Carbondale, Illinois 62901-6501
[2]Fisheries and Aquaculture Center, Department of Zoology,
Southern Illinois University, Carbondale, Illinois 62901-6501

ABSTRACT

The cotton mouse (*Peromyscus gossypinus*) is on the northern periphery of its range in southwestern Kentucky, southeastern Missouri, and southern Illinois. Little information is available on the life history of cotton mice in Illinois, in part because of difficulty in differentiating them from white-footed mice (*P. leucopus*). Current identification is often based on lethal sampling, including collection of internal tissues for allozyme electrophoresis or measurement of skull characters. Here we describe a reliable, non-lethal method for distinguishing between cotton mice and white-footed mice using a diagnostic allozyme locus, glucose-6-phosphate isomerase (GPI-1) from toe-clips. This technique will enhance conservation efforts by making identification of *P. gossypinus* and *P. leucopus* easier in areas of sympatry.

INTRODUCTION

The cotton mouse was first described by LeConte in 1853 as *Hesperomyscus gossypinus* (LeConte, 1853; Bangs, 1896). Osgood (1909) revised the taxonomy and recognized four subspecies based on size and pelage colorations: *P. g. anastasae*, *P. g. gossypinus*, *P. g. megacephalus*, and *P. g. palmarius*. A large, pale subspecies, *P. g. megacephalus*, occurs in southern Illinois, southwestern Kentucky, and southeastern Missouri (Hoffmeister, 1989).

Cotton mice are one of the most abundant mammalian species in the southeastern United States (Pournelle, 1952). Their geographic range extends from southeastern Virginia, south through Florida, west to eastern Texas, and north through Tennessee to western Kentucky (Hoffmeister, 1989). The species is on the northern edge of its range in southern Illinois, the Jackson Purchase Region of Kentucky (Barbour and Davis, 1974), and southeast Missouri, including the bootheel region (Hall, 1981). The cotton mouse is not listed as threatened or endangered in Illinois, but is a species of concern in Missouri (Bekiares, 2000) and threatened in Kentucky (Kentucky State Nature Preserves Commission, 1998; Bekiares, 2000).

The cotton mouse is sympatric with the white-footed mouse (*P. leucopus*) in Arkansas, Louisiana, Mississippi, western Tennessee, northern Alabama, and in portions of Georgia, South Carolina, North Carolina, Virginia, Kentucky, Illinois, and Missouri (Hall, 1981; Robbins et al., 1985; Hoffmeister, 1989). Sympatry among species of *Peromyscus* is common in many geographic areas (Sternburg and Feldhamer, 1997) and identification often is difficult because of morphological similarity (Wolfe and Linzey, 1977; Schwartz and Schwartz, 1981; Engstrom et al., 1982; McDaniel et al., 1983). In Missouri, the reported range of the hindfoot length (HF) of adult cotton mice is 20-25 mm; the range of body mass (BM) is 19-25 g (Schwartz and Schwartz, 1981). These values overlap ranges reported for white-footed mice (HF = 19-25 mm; BM = 11-28 g). In Kentucky, the hindfoot length of cotton mice (HF = 21-26 mm) overlaps the range reported by Barbour and Davis (1974) for white-footed mice (HF = 19-22 mm). Ranges reported by Hoffmeister (1989) for cotton mice (HF = 22-25 mm) and white-footed mice (HF = 18-22 mm) in Illinois also overlap.

Methods used to distinguish sympatric species of *Peromyscus* include adrenal weight (Christian, 1967), calcaneum size (Stains, 1959), ratios of morphological characteristics (Hoffmeister, 1977), red blood cell immune agglutination (Moody, 1941), karyotyping (Hsu and Arrighi, 1966; Pathak et al., 1973), and genic variation using electrophoresis (Price and Kennedy, 1980; Palas et al., 1992). Many of these techniques are time consuming, expensive, and/or involve sacrificing animals, which may not be practical for ecological, conservation, and/or behavioral studies (Feldhamer et al., 1983).

Our objective was to determine a non-lethal method for distinguishing between cotton mice and white-footed mice that would make future identification easier, more reliable, and of use in conservation projects where euthanasia of animals for identification purposes is unacceptable. We compared a non-lethal laboratory electrophoresis procedure using tissues obtained from toe-clips with a validated lethal electrophoresis technique using liver tissue (Price and Kennedy, 1980). Furthermore, we compared the electrophoresis results with a morphological technique based on a scatter diagram of skull and hindfoot measurements developed by Hoffmeister (1977).

MATERIALS AND METHODS

Collection of *Peromyscus* samples for analysis was conducted during November 1997 in New Madrid Co., Missouri, in bottomland hardwood forested areas located in Donaldson Point State Forest. We used Sherman live traps (8 x 9 x 23.5 cm), baited with cracked corn and sunflower seeds, and Museum Special snap traps, baited with peanut butter. Traps were set in the afternoon along transects, with traps placed 10 m apart. Traps were operated for a total of 730 trap nights. All animals with a hindfoot length ≥ 22 mm or a body mass ≥ 26 g were considered potential cotton mice based on ranges of morphological features (Barbour and Davis, 1974; Schwartz and Schwartz, 1981; Hoffmeister, 1989; Feldhamer et al., 1998). Live-trapped potential cotton mice were euthanized. Snap trapped and euthanized live-trapped *Peromyscus* were wrapped in aluminum foil and placed on dry ice for transport to the laboratory.

In the laboratory, sex, reproductive condition, and age were recorded, and body mass, hindfoot length, total body length, and tail length were measured. Toe-clips and internal

tissues (liver and muscle) were collected and placed in separate microcentrifuge tubes. An approximately equal volume of grinding buffer (a mixture of 2% 2-phenoxyethanol and 0.25 M sucrose; see Nakanishi et al., 1969) was added to each tube and the tissue samples were frozen at -70°C for future genetic analysis (Hillis et al., 1996). Skulls were cleaned with dermestid beetles (*Dermestes vulpinus*) to measure length of nasals, condylobasal length, and crown length of maxillary toothrow. All abbreviations for enzymes follow Shaklee et al. (1990) and all names and enzyme commission numbers follow IUBNC (1984).

Morphological identification of cotton mice and white-footed mice was based on a scatter diagram developed by Hoffmeister (1977). Condylobasal length multiplied by the maxillary toothrow was plotted against the hindfoot length multiplied by the length of the nasals. This technique was compared with the results of our non-lethal genetic technique.

Price and Kennedy (1980), using starch-gel electrophoresis, found glucose-6-phosphate isomerase (GPI-1; EC 5.3.1.9) exhibited diagnostic alleles between *P. gossypinus* and *P. leucopus* when using internal tissues (lethal sampling). We attempted to isolate this allozyme from toe-clips and verify the banding using internal tissue (i.e., liver). Non-lethal sampling often yields a lower quality of enzyme extracts. Therefore, we employed cellulose acetate (CA) electrophoresis as described by Hebert and Beaton (1993). This technique requires smaller amounts of enzyme than starch gel electrophoresis.

Before conducting allozyme electrophoresis, 80 μl of distilled water was added to each sample. Tissue samples were homogenized in the microcentrifuge tubes with a disposable pestle (Kimble Sciences Products, Vineland, NJ). Homogenates were centrifuged at approximately 10,000 G's for five minutes in order to separate the supernatant (with enzymes) from cellular debris.

Ten μl of the resulting supernatant was placed in an individual loading plate well (Helena Laboratories, Beaumont, TX). Toe-clip and liver samples from the same individual were run to ensure enzyme quality/quantity from toe-clips. Six individuals (12 lanes) were run at a time. A continuous Tris Glycine (pH 8.5) buffer system was used. Gels were electrophoresed at 191 v for 25 min. Following electrophoresis, gels were histochemically stained, scored, dried in an oven, and saved as vouchers (Hebert and Beaton, 1993).

RESULTS

Twenty-eight *Peromyscus* meeting the criteria of hind foot length or body mass were removed from the field. Four cotton mice were identified using mensural characteristics (Fig. 1) based on Hoffmeister (1977). The remaining 24 *Peromyscus* were white-footed mice. The mean body mass of adult cotton mice was 34.8 g (SD = 0.42). This mass was different ($t = 2.53$, $p < 0.01$, $df = 21$) from the mean body mass of adult white-footed mice (28.8 g , SD = 3.28). Mean hindfoot length (24 mm; SD=0.00) of adult cotton mice was greater ($t = 4.94$, $p < 0.0005$, $df = 21$) than the mean hind foot length (20.91, SD = 0.89 mm) of adult white-footed mice.

We verified that *GPI-1** was a diagnostic locus, and identical banding was produced using tissue from liver and toe-clips (Fig. 2). The allelic mobility was significantly faster

(more cathodal) in cotton mice when compared to the allelic mobility of white-footed mice. Three of the 4 cotton mice identified using Hoffmeister (1977) were identified using this non-lethal diagnostic allozyme marker (Fig. 1).

DISCUSSION

The morphological measurements of cotton mice in our study represented the maxima in the range of measurements reported in the tristate area (Barbour and Davis, 1974; Schwartz and Schwartz, 1981; Hoffmeister, 1989), and were similar to the means of cotton mice recently collected in Kentucky (BM = 32.87; HF = 22.75) and Missouri (BM = 28.68; HF = 23.5) by Bekiares (2000.). Feldhamer et al. (1998) reported the average hindfoot length and body mass of Illinois cotton mice were 22.4 mm and 26.7 g, respectively. All adult cotton mice in our study and Feldhamer et al. (1998) adhered to the "general rule" of body mass ≥ 26 g or hindfoot length ≥ 22 mm as well as the ratios established by Hoffmeister (1989). However, all cotton mice did not exhibit both morphological characteristics.

Our findings suggest that although morphological measurements may indicate a potential cotton mouse, additional methodology is needed for positive species identification (i.e., allozyme electrophoresis). Based on morphological measurements alone, we would have misidentified 17 white-footed mice as cotton mice because they met one or both of the hindfoot and body mass criteria. Additional factors, such as reproductive condition and age of the individual, can make identification based on these measurements difficult.

Boone (1995) suggests cotton mice exhibit a clinal geographic pattern, with larger individuals on the northeastern, northwestern, and southwestern edges of their range. The mice collected in Missouri adhered to this pattern in that they were relatively large. Bekiares (2000) also found large individuals in Missouri and Kentucky. However, Feldhamer et al. (1998) found small individuals in Illinois. These findings reinforce the need for a reliable method of species identification in this tristate area.

The use of toe-clips and allozyme electrophoresis for species identification is useful because toe-clips are commonly taken during small mammal studies for mark/recapture information. Toe-clips are also taken in studies that involve animal movements, species abundance/evenness estimation, and long-term population monitoring. The removal of a toe-clip has minimal effect on an individual.

In Illinois, this non-lethal technique is especially useful because the status of the cotton mouse is not known. The species was not reported in Illinois for nearly 90 years (Hoffmeister, 1989), until they were captured in 1996 at Horseshoe Lake Conservation Area, Alexander Co. (Feldhamer et al., 1998). Little information is available on the life history of cotton mice in Illinois, in part because of past difficulty in species identification. Our method of distinguishing between cotton mice and white-footed mice will enhance conservation efforts by simplifying future identification of these species in areas of sympatry and provides an alternative method for use in projects where euthanasia of animals for identification purposes is unacceptable.

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Figure 1. Scatter diagram of two ratios used to separate *P. leucopus* and *P. gossypinus* (based on Hoffmeister, 1977). Individuals to the right of the line are presumed *P. gossypinus* and those to the left of the line are presumed *P. leucopus*. The three largest individuals (upper right corner) were identified as *P. gossypinus* by the scatter diagram and our non-lethal allozyme marker.

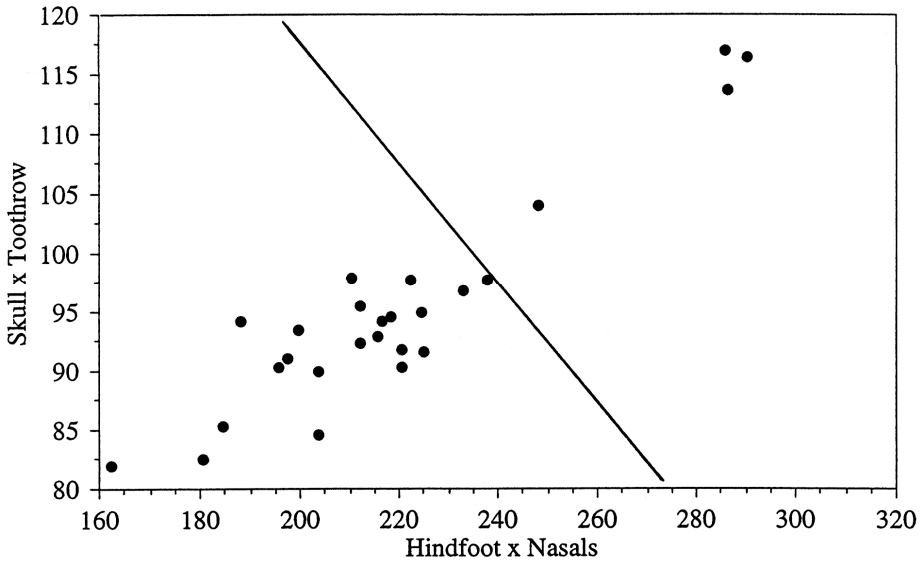

Figure 2. Allelic mobility of P. *leucopus* and *P. gossypinus* and banding patterns of toe-clip (lane 12) and liver tissue (lane 5) of *P. gossypinus* at the *GPI-1** locus.