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Cotton Mice (Peromyscus gossypinus) in Southern Illinois: Evidence for Hybridization with White-footed Mice (Peromyscus leucopus)

VALERIE A. BARKO[1] AND GEORGE A. FELDHAMER
Department of Zoology, Southern Illinois University, Carbondale 62901

ABSTRACT.—We sampled sixty bottomland forest patches in the six southwestern-most counties in Illinois to determine the current status of the cotton mouse (*Peromyscus gossypinus*). Identification of *Peromyscus* was based on a modified allozyme electrophoretic technique with the diagnostic *GPI-1** locus. Allozymes were isolated from toe-clip samples, rather than liver, and run on a cellulose acetate medium. One hybrid *Peromyscus gossypinus-leucopus* and one small *Peromyscus*, carrying a cotton mouse allele at the *GPI-1** locus, were identified from 384 individuals screened with this genetic marker. We suggest that cotton mice are an ephemeral species in southern Illinois, disperse into the area occasionally or only during extreme environmental changes and breed with available white-footed mice because of small population size and reduced mate choice.

INTRODUCTION

The cotton mouse (*Peromyscus gossypinus*) is a large woodland species on the northern periphery of its range in southern Illinois, southeastern Missouri and the Jackson Purchase region of Kentucky (Hoffmeister, 1989). Its geographic range extends from southeastern Virginia, south through Florida, west to eastern Texas and north through Tennessee to western Kentucky. In Illinois the cotton mouse was historically distributed south of the Ozark Plateau and Shawnee Hills and was reported in the five southwestern-most counties of Illinois (Hoffmeister, 1989).

Cotton mice mainly inhabit swampy woodlands and adjacent forests in the southeastern United States (Barbour and Davis, 1974; Wolfe and Linzey, 1977; Hoffmeister, 1989; Laerm and Boone, 1994). However, this species also occurs in bottomland forests, near oxbow lakes and areas with a high water table (McCarley, 1954, 1963; Bradshaw, 1968; Laerm and Boone, 1994).

The cotton mouse had not been reported in Illinois since 1909 despite ample sampling over the past 50 y specifically to locate them (Feldhamer *et al.*, 1998). In 1996 five cotton mice (identification based on morphology) were collected from Horseshoe Lake Conservation Area, Alexander Co., in extreme southwestern Illinois (Feldhamer *et al.*, 1998). Other individuals (n = 12) presumed to be cotton mice based on body mass and morphology were trapped and released.

Identification of cotton mice generally is problematic because of their morphological similarity to sympatric species of *Peromyscus* found in Illinois (Linzey *et al.*, 1976; Hoffmeister, 1989), including the white-footed mouse (*P. leucopus*) and deer mouse (*P. maniculatus*). In Illinois the reported range of hind foot length (HF) of adult cotton mice is 22–25 mm (Hoffmeister, 1989). This range slightly overlaps that reported for white-footed mice (HF = 18–22 mm), which slightly overlaps the range reported for deer mice (HF ≤ 18 mm; Hoffmeister, 1989). Identification of *Peromyscus* is usually based on mensural char-

[1] Present address: Missouri Department of Conservation, Open River Field Station, 3815 E. Jackson Blvd., Jackson, Missouri 63755

acteristics (Hoffmeister, 1977; Laerm and Boone, 1994) or allozyme electrophoresis (Price and Kennedy, 1980; Robbins *et al.*, 1985; Sternburg and Feldhamer, 1997). Based on body mass and ranges in hind foot length of cotton mice reported from the northern periphery of the range (Kentucky: Barbour and Davis, 1974; Missouri: Schwartz and Schwartz, 1981; Illinois: Hoffmeister, 1989; Feldhamer *et al.*, 1998), we used a ''general rule'' for identifying a potential cotton mouse as hind foot length ≥22 mm and/or body mass ≥26 g. Other studies have reported hind foot length as the most useful morphological character in distinguishing between cotton mice and white-footed mice (Dice, 1940; McCarley, 1954).

Blair (1950) suggested the potential for hybridization exists in all congeneric vertebrate groups. Natural hybrids (identification based on morphology) between *Peromyscus gossypinus* and *P. leucopus* have been reported from areas of sympatry (Howell, 1921; McCarley, 1954; St. Romain, 1974; Lovecky *et al.*, 1979). Dice (1937, 1940) reported hybrids of these species are completely interfertile when crossed with each other and when backcrossed with their parental species. Morphological characters of cotton mouse × white-footed mouse hybrids are intermediate in size (Dice, 1940; but *see* Bradshaw, 1968).

It is unknown whether cotton mice have been in southern Illinois since 1909 and have simply been misidentified. Conversely, this species may have returned to the area in association with pronounced environmental changes such as the large-scale flooding in 1993 and 1994 (Bhowmik *et al.*, 1994). Alternatively, small ephemeral cotton mouse populations may occur, but either hybridize with the more abundant white-footed mice or become extirpated.

Our objectives were to: (1) determine the current distribution of *Peromyscus gossypinus* in southern Illinois, and (2) determine whether *P. gossypinus* and *P. leucopus* hybridize in southern Illinois.

MATERIALS AND METHODS

Study sites were located by use of an ArcView Geographic Information System (GIS) v.3.× (Environmental Systems Research Institute, New Jersey). Five spatial coverages from Illinois Natural History Survey data and Illinois State Geological Survey data were combined to identify suitable cotton mouse habitat in the six southwestern-most counties of Illinois (Alexander, Johnson, Massac, Pope, Pulaski and Union; *see* Barko, 2000). We considered ''suitable habitat'' to be hardwood bottomland forest, a minimum size of 8 ha and ≥100 m from a primary or secondary road. This 100-m buffer was established because cotton mice are not considered an ''edge'' species. Nondeciduous or upland habitats were not surveyed based on the findings of Schmid (1998) who surveyed eighty sites in these habitats and captured no cotton mice. Sixty study sites were chosen based on landowner permission and water levels. A Magellin Trailblazer XL Global Positioning System (GPS) was used to accurately determine the location of each study site (*see* Barko, 2000).

Animals were captured from May 1998 through August 1999 by use of Sherman live traps (8 cm × 9 cm × 23.5 cm) set in a standardized transect approximately 500 m in length. One hundred traps were set at each site, with trap stations placed 10 m apart. Traps were set in the afternoon near fallen logs, stumps, water body edges or tree trunks to maximize cotton mouse trap success (Boone *et al.*, 1993; McCay, 2000). Two traps were set at each station and baited with sunflower seeds and cracked corn. Traps were covered with organic debris to reduce exposure to direct sunlight. During cold weather polyester fiberfill was placed in each trap. Odor baiting was not used and traps that captured animals were disinfected before placement at a new site (*see* Millis *et al.*, 1995). Traps were operated for 3 consecutive days (300 trap nights per site) and examined daily between 0600 and 1100 h. Individual animals were toe-clipped for identification and allozyme electrophoresis, and

hind foot length, body mass, sex and reproductive condition were recorded (Feldhamer *et al.*, 1983; Hoffmeister, 1989; Sternburg and Feldhamer, 1997). All animals were released at the point of capture.

Toe-clip samples were placed in separate microcentrifuge tubes and 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. Tubes were stored on icepacks in a soft-sided cooler until return to the laboratory to prevent denaturing of proteins (Manlove *et al.*, 1975). Toe-clip samples were then frozen at −70 C for future allozyme analysis (Hillis *et al.*, 1996).

Barko *et al.* (2000) verified that glucose-6-phosphate isomerase (*GPI-1**; EC 5.3.1.9), from toe-clip samples, exhibited diagnostic alleles between *Peromyscus gossypinus* and *P. leucopus* (*see* Price and Kennedy, 1980; Robbins *et al.*, 1985). This eliminated the need for the use of internal tissue (liver) and the necessity of sacrificing individual animals. We took a conservative approach because the cotton mouse is listed as an endangered species in Kentucky (Kentucky Nature Preserves Commission, 1998; Bekiares, 2000), a species of concern in Missouri (Bekiares, 2000), and is of unknown status in Illinois (Hoffmeister, 1989; Feldhamer *et al.*, 1998).

Cellulose acetate (CA) electrophoresis was conducted on toe-clip samples from potential cotton mice (hindfoot ≥22 mm and/or body mass ≥26 g) and a random sample (25%) of the remaining mice. A standard was placed on every gel which was a known cotton mouse from Kentucky (*see* Bekiares, 2000). All abbreviations for enzymes follow Shaklee *et al.* (1990) and all names and enzyme commission numbers follow IUBNC (1984).

Unpaired *t*-tests were used to compare hind foot length and body mass of cotton mice recently captured in Kentucky, Illinois and Missouri by Feldhamer *et al.* (1998), Bekiares (2000) and Barko *et al.* (2000). Because morphological characteristics of mice captured by Feldhamer *et al.* (1998) in Illinois were small, we were unsure if individuals were cotton mice or natural hybrids. Only adults, based on pelage coloration and body mass (>18 g) were used in analyses (Cummings and Vessey, 1994; Nupp and Swihart, 2000) and significance was $\alpha = 0.05$ (Steel and Torrie, 1980). Voucher specimens (SIU # 4307–4333) were deposited in the Mammal Museum, Southern Illinois University, Carbondale.

RESULTS

A total of 1309 *Peromyscus* sp. were captured and toe-clipped during 18,000 trap nights (trap success rate = 7.3%). One-hundred eighteen mice were screened at the diagnostic *GPI-1** locus as potential cotton mice (hind foot length ≥22 mm and/or body mass ≥26 g) and 266 mice were screened at the same locus to verify that they were white-footed mice (random sampling of 25%). One individual was identified as a hybrid (body mass = 22 g), based on a heterozygous GPI-1 marker. One mouse from the random sampling (hind foot length = 21 mm; body mass = 18.5 g) was homozygous for the cotton mouse allele. All other screened mice (382 individuals) were *P. leucopus* based on electrophoretic results. We determined that the remaining 925 individuals were white-footed mice based on morphology and electrophoretic results of the random sample.

DISCUSSION

The ''general rule'' for identifying cotton mice (hind foot length ≥22 mm or body mass ≥26 g; Hoffmeister, 1989) did not enable us to accurately identify cotton mice in Illinois. One-hundred eighteen mice had one or both of these criteria and none were cotton mice based on genetic testing. The individuals with the homozygous cotton mouse and hetero-

zygous alleles had morphological measurements within the range reported for white-footed mice, and would have been misidentified without genetic testing.

We suggest that *Peromyscus gossypinus* and *P. leucopus* hybridize in southern Illinois. The small cotton mouse and hybrid *Peromyscus* we identified using the *GPI-1** marker could have been back-crossed with *P. leucopus*. This would explain the small hind foot length and low body mass of both individuals. Backcrossing often masks morphological differences between the species (McCarley, 1954). One disadvantage of the nonlethal technique we used is that only one locus was examined. Because multiple loci were not examined, we had a 50% probability of misidentifying a hybrid. A f_x-individual could have the *P. leucopus* allele at the *GPI-1** locus, but *P. gossypinus* alleles at other loci. Also, we were not able to distinguish between a f_1-hybrid and a f_x-hybrid. Both could exhibit the *P. gossypinus* allele at the *GPI-1** locus. However, this technique did allow us to identify a hybrid individual and indicates the conservative or minimum level of hybridization in southern Illinois between *P. leucopus* and *P. gossypinus*.

Further support for hybridization between *Peromyscus gossypinus* and *P. leucopus* in southern Illinois is suggested by the results of Feldhamer *et al.* (1998). They identified five cotton mice (*P. gossypinus megacephalus*) at Horseshoe Lake Conservation Area, Alexander Co., in 1996. Their identification was based on the mensural characteristics of Hoffmeister (1977) and two discriminant function equations of Laerm and Boone (1994). Feldhamer *et al.* (1998) did not consider an individual a cotton mouse unless both methods established it as such. However, genetic analyses were not conducted and presumptive cotton mice often fell along the scattergram line of Hoffmeister (1977) separating *P. leucopus* and *P. gossypinus*. Cotton mice captured in Illinois by Feldhamer *et al.* (1998) generally were smaller, based on average hind foot length (MO: $t = 2.85$, df = 6, $P < 0.01$; MO/KY: $t = 0.89$, df = 43, $P > 0.05$) and average body mass (MO: $t = 7.46$, df = 6, $P < 0.001$; MO/KY: $t = 5.67$, df = 43, $P < 0.001$), than cotton mice in Missouri (Barko *et al.*, 2000; Bekiares, 2000) and Kentucky (Bekiares, 2000). There were no statistically significant differences between the average of hind foot length ($t = 1.60$, df = 40, $P > 0.05$) and body mass ($t = 0.70$, df = 40, $P > 0.2$) of the Missouri and Kentucky cotton mice.

In a recent study in Kentucky and Missouri, Bekiares (2000) tested the methods of Hoffmeister (1977) with allozyme electrophoresis at several loci, including the diagnostic *GPI-1** locus. She identified individuals on or near the scattergram line as white-footed mice. This suggests the mice identified by Feldhamer *et al.* (1998) could be hybrids, based on their scattergram position using the criteria of Hoffmeister (1977). Four of the five specimens likely had cotton mice alleles because mesostylids were present (*see* Hoffmeister, 1977).

Additional evidence for hybridization in southern Illinois cotton mice is provided by comparing the means of body mass and hind foot length of specimens captured in Illinois, Missouri and Kentucky. Feldhamer *et al.* (1998) reported significant differences in average measurements between presumptive cotton mice and white-footed mice from Horseshoe Lake Conservation Area. Nonetheless, the cotton mice in Illinois were significantly smaller compared with those of adult *Peromyscus gossypinus megacephalus* in Kentucky and Missouri (Bekiares, 2000; Barko *et al.*, 2000). These findings agree with Bradshaw (1968), who reported that hybrids of cotton mice and white-footed mice had morphological characters intermediate in size.

We suggest that the cotton mouse is an ephemeral species in southern Illinois and may disperse into the area only occasionally. The few immigrants into Illinois may hybridize with *Peromyscus leucopus*. Although cotton mice prefer to breed with conspecifics, they will breed with white-footed mice if there is limited mate choice (McCarley, 1964; Bradshaw, 1965).

McCarley (1964) reported a lack of mate choice between allopatric cotton mice and white-footed mice when breeding wild-caught individuals in the laboratory. However, strong intraspecific mate choice was exhibited in sympatric populations of cotton mice and white-footed mice. The probability of encountering another cotton mouse might be low during extreme environmental conditions or at the periphery of their range because conspecifics are rare or absent.

McCarley (1963) studied habitat relationships between sympatric species of cotton mice and white-footed mice. He reported white-footed mice inhabit both upland and bottomland forested areas in areas of allopatry. However, white-footed mice are found mainly in upland areas when they are sympatric with cotton mice (McCarley, 1963), which occur mainly in bottomland forests. McCarley (1963) concluded cotton mice prevent white-footed mice from inhabiting bottomland forests in areas of sympatry, and create allotopic distribution patterns. This is consistent with our suggestion that cotton mice are ephemeral in southern Illinois. All 1307 individual white-footed mice we captured were in bottomland hardwood forests. There is little to no competitive exclusion by cotton mice because they are rare or absent and white-footed mice inhabit both bottomland and upland areas (Hoffmeister, 1989; Schmid, 1998).

All of the cotton mice identified in Illinois since 1996 have been in the extreme southwestern portion of the state in Alexander and Union counties. This distribution is consistent with large-scale flooding (Bhowmik *et al.*, 1994). We suggest cotton mice recently re-entered southern Illinois with flood waters from the Mississippi River, at the convergence with the Ohio River at Cairo, Illinois. Individuals probably dispersed into Illinois from Kentucky, which is the closest known population. It is probable that some of these cotton mice bred with white-footed mice because of small population size and reduced mate choice. This is a plausible explanation for our results: two small *Peromyscus*, one with a cotton mouse allele and a hybrid with both a cotton mouse and white-footed mouse allele. It may also explain why the cotton mice captured by Feldhamer *et al.* (1998) were significantly smaller than those captured in nearby Missouri and Kentucky.

Our evidence suggests that southern Illinois is a possible hybrid zone between *Peromyscus gossypinus* and *P. leucopus*. Intensive genetic screening of *Peromyscus* should be conducted in southern Illinois, to further document this potential hybrid zone.

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