

**FINAL REPORT**

**"Identification, current distribution, and relative abundance of  
the cotton mouse in Illinois"**

**WPF Small Project No. FY00-012**

**Submitted to:**

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**It includes only those chapters dealing with identification, distribution, and relative abundance of cotton mice, i.e. Chapters 2 and 3 (pages 14-59) and the Literature Cited and Appendices (pages 85-112)**

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CHAPTER 2 - A NON-LETHAL METHOD FOR IDENTIFICATION  
OF THE COTTON MOUSE, PEROMYSCUS GOSSYPINUS

INTRODUCTION

The cotton mouse was first described by LeConte in 1853 (LeConte, 1853; Bangs, 1896) as Hesperomyscus gossypinus. Osgood (1909) revised the taxonomy and recognized four subspecies based on size and pelage coloration: 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; Figure 2.1). In Illinois, the cotton mouse was historically distributed south of the Ozark Plateau and Shawnee Hills (Hoffmeister, 1989) and was reported in Alexander, Johnson, Pope, Pulaski, and Union Counties (Hoffmeister 1989; Figure 2.2).

The cotton mouse is on the northern periphery of its

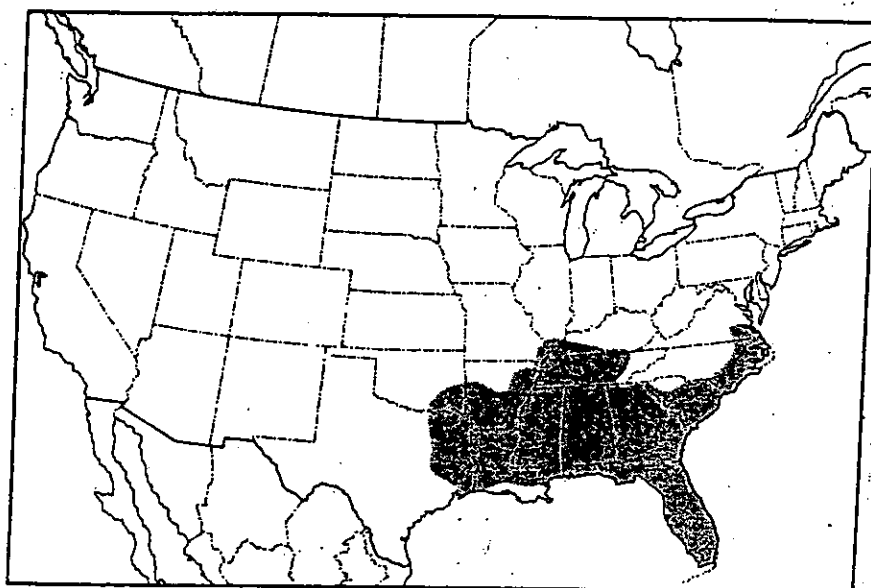


Figure 2.1. Geographic range of the cotton mouse (Peromyscus gossypinus); Hoffmeister, 1989.

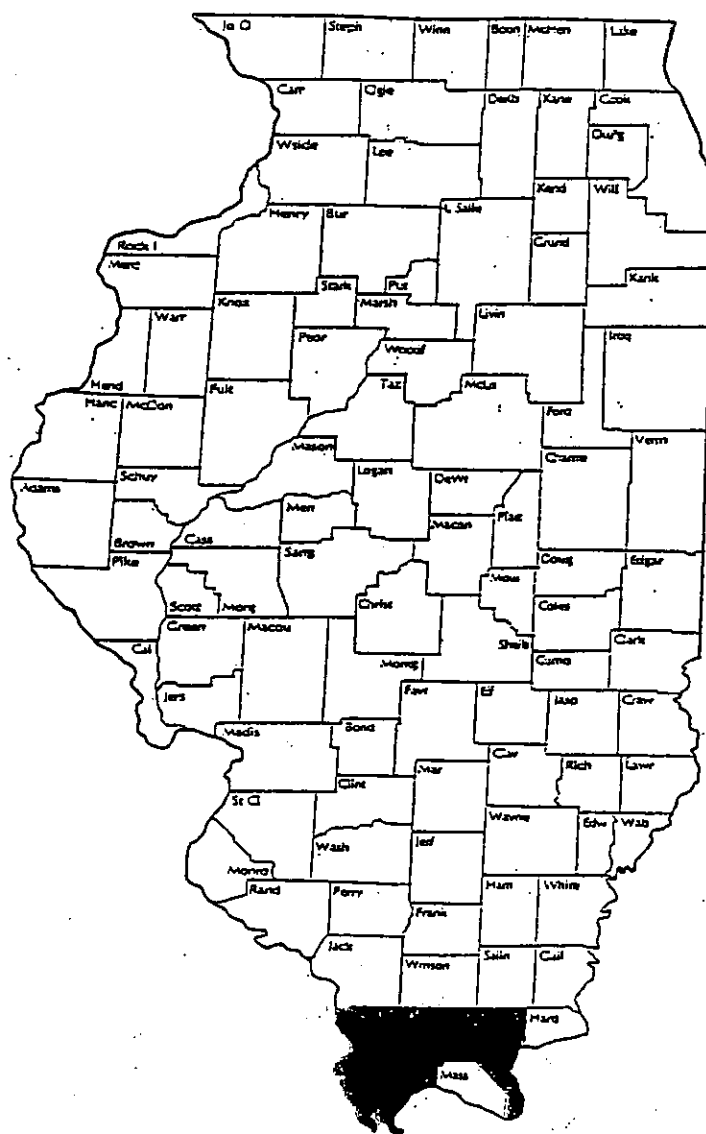


Figure 2.2. Historical distribution of the cotton mouse (*Peromyscus gossypinus*) in Illinois (Hoffmeister, 1989).

range in southern Illinois, the Jackson Purchase Region of Kentucky (Barbour and Davis, 1974), and southeast Missouri, including the bootheel region (Hall, 1981). This species is not listed as threatened or endangered in Illinois, but is a species of concern in Missouri (Bekiaries, 2000) and threatened in Kentucky (Kentucky State Nature Preserves Commission, 1998; Bekiaries, 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; Figure 2.3). 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

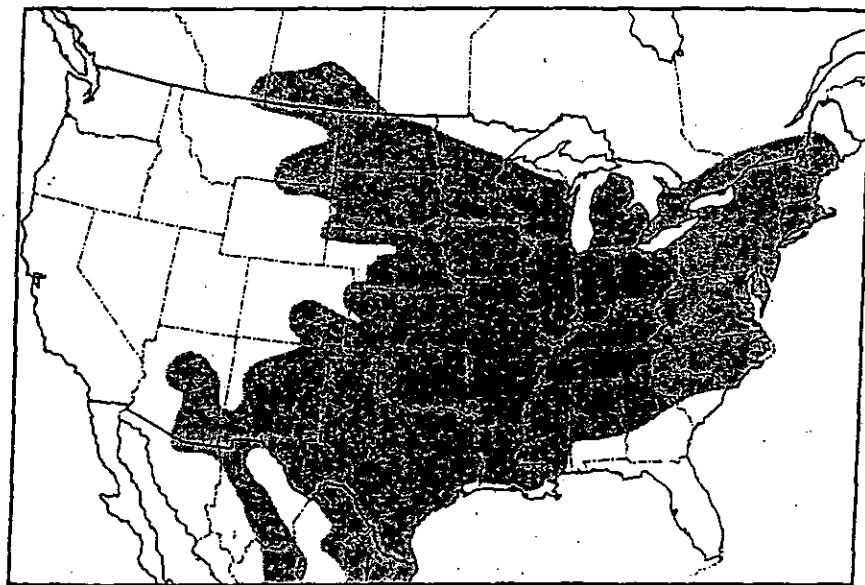


Figure 2.3. Geographic range map of the white-footed mouse (Peromyscus leucopus); Hoffmesiter, 1989.

range reported for white-footed mice (HF = 19-22 mm; Barbour and Davis, 1974). 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 involve sacrificing animals, which may not be practical for ecological, conservation, and/or behavioral studies (Feldhamer et al., 1983).

My objective was to determine a reliable, nonlethal 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. I compared a non-lethal laboratory electrophoresis technique using tissues obtained from toe-clips with a validated lethal electrophoresis technique



using liver tissue (Price and Kennedy, 1980). Furthermore, I 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

### Field Sampling

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. I used Sherman live traps (8 x 9 x 23.5 cm; H.B. Sherman Co., Florida), 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  $\geq$  22 mm or a body mass  $\geq$  26 g were considered potential cotton mice based on ranges of morphological features noted previously (Barbour and Davis, 1974; Schwartz and Schwartz, 1981; Hoffmeister, 1989; Feldhamer et al., 1998). Potential cotton mice were euthanized, wrapped in aluminum foil, and placed on dry ice for transport to the laboratory. All snap trapped

Peromyscus were also transported to the laboratory.

#### Voucher and Tissue Preparation

In the laboratory, sex, reproductive condition, and age was 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  $-70^{\circ}\text{C}$  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 scattergram 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 is

commonly used to differentiate between the species (see Feldhamer et al., 1998) and was compared with our non-lethal genetic technique. I identified Peromyscus using the results of the allozyme marker.

#### Allozyme Electrophoresis

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). I 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, I 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  $\mu$ l 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  $\mu$ l of the resulting supernatant were placed in an individual loading plate well (Helena Laboratories, Beaumont, TX). Toe-clips 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 (Appendix 1). Gels were electrophoresed at 191 volts for 25 minutes. Following electrophoresis, gels were histochemically stained, scored, dried in an oven, and saved as vouchers (Hebert and Beaton, 1993; Appendix 1).

#### Data Analysis

Descriptive statistics were calculated (mean, standard deviation, minimum, maximum) for all morphological and skull measurements. Unpaired t-tests were calculated to compare means and  $\alpha = 0.05$ . Only adults were used in the analysis of morphometric data. A scattergram was also created, based on Hoffmeister (1977), to differentiate between cotton mice and white-footed mice.

## RESULTS

### Peromyscus Captures

Twenty-eight Peromyscus meeting the criteria of hind foot length or body mass were removed from the field. From these, four cotton mice were identified using mensural characteristics (Figure 2.4) based on Hoffmeister (1977). The remaining 24 Peromyscus were identified as white-footed mice.

### Morphometric Variation

Eight morphometric traits were examined (body mass, hindfoot length, ear length, tail length, total length, skull length (condylobasal length), length of nasal bones, and maxillary toothrow length) in both cotton mice and white-footed mice (Table 2.1). Cotton mice were larger in all traits (Table 2.2), except tail length and length of the nasals. The means of these traits were not different between cotton mice and white-footed mice.

Using the same eight morphometric traits of cotton mice, means were compared to means of other cotton mice captured in Kentucky and Missouri (Bekiaries, 2000; Table 2.3). The only trait that was different was the length of

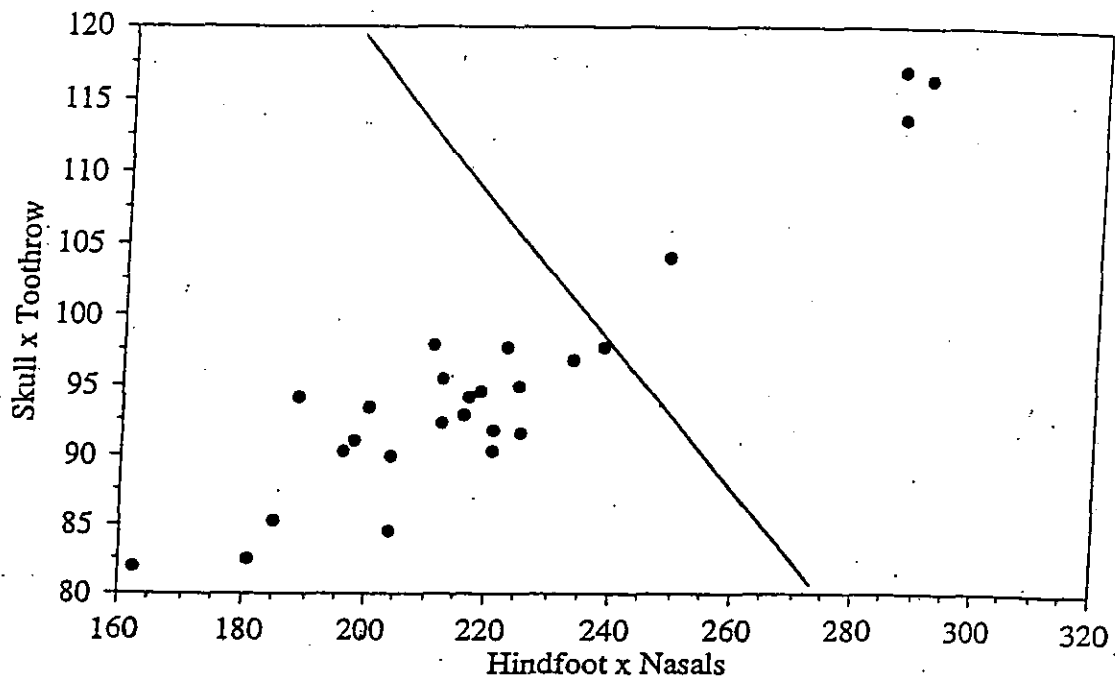


Figure 2.4. Scatter diagram of two ratios (hindfoot length x nasal length and skull length x maxillary toothrow length) used to separate *P. gossypinus* and *P. leucopus* (based on Hoffmeister, 1977). Individuals to the right of the scattergram line are presumed cotton mice and individuals to the left are presumed white-footed mice. The three largest individuals (top right) were identified as cotton mice using the genetic marker.

Table 2.1. Summary of Peromyscus morphometric measurements for cotton mice and white-footed mice captured in New Madrid County, Missouri in November 1997.

<u>Measurement</u>	<u>n</u>	<u>Mean</u>	<u>Min.</u>	<u>Max.</u>
<b>Body Mass (g)</b>				
<u>P. gossypinus</u>	2	34.8	34.5	35.1
<u>P. leucopus</u>	21	28.8	21.8	33.5
<b>Total Body Length (mm)</b>				
<u>P. gossypinus</u>	2	181.5	172.0	191.0
<u>P. leucopus</u>	20	164.1	144.0	184.0
<b>Tail Length (mm)</b>				
<u>P. gossypinus</u>	2	78.5	72.0	85.0
<u>P. leucopus</u>	20	70.4	50.0	81.0
<b>Hindfoot Length (mm)</b>				
<u>P. gossypinus</u>	2	24.0	24.0	24.0
<u>P. leucopus</u>	21	20.9	19.0	22.0
<b>Ear Length (mm)</b>				
<u>P. gossypinus</u>	2	17.0	16.0	18.0
<u>P. leucopus</u>	20	14.0	9.0	17.0

Table 2.1. Continued.

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<u>Measurement</u>	<u>n</u>	<u>Mean</u>	<u>Min.</u>	<u>Max.</u>
<u>Nasals (mm)</u>				
<u>P. gossypinus</u>	2	12.0	11.9	12.1
<u>P. leucopus</u>	20	10.1	9.4	10.8
 <u>Skull Length (mm)</u>				
<u>P. gossypinus</u>	2	29.6	29.1	30.0
<u>P. leucopus</u>	20	25.9	24.4	27.8
 <u>Maxillary Toothrow (mm)</u>				
<u>P. gossypinus</u>	2	4.0	3.9	4.0
<u>P. leucopus</u>	20	3.6	3.3	3.8

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Table 2.2. Comparison of morphometric and mensural characters between Peromyscus gossypinus and P. leucopus captured in New Madrid County, Missouri in November 1997.

SPECIES	BODY MASS (g)	HINDFOOT (mm)	EAR LENGTH (mm)	TAIL LENGTH (mm)	TOTAL BODY LENGTH (mm)	SKULL LENGTH	NASALS (mm)	MAXILLARY TOOTHROW (mm)
<u>Peromyscus gossypinus</u>								
Mean	34.80	24.00	17.00	78.50	181.50	29.55	12.00	3.95
S.D.	0.42	0.00	1.41	9.19	13.44	0.64	0.14	0.07
n = 2								
<u>Peromyscus leucopus</u>								
Mean	28.80	20.91	13.95	70.43	164.05	25.93	10.14	3.59
S.D.	3.28	0.09	2.19	7.17	11.55	0.94	0.44	0.02
n = 21								
<u>Statistics</u>								
t	2.53	4.94	1.91	1.49	2.02	5.30	1.29	4.10
P	**	***	*	**	*	***	x	***
df	21.00	21.00	20.00	20.00	20.00	20.00	20.00	20.00

$P > 0.05 = x$

$P < 0.05 = *$

$P < 0.01 = **$

$P < 0.001 = ***$

Table 2.3. Comparison of cotton mice morphometric and mensural characteristics in this study and Bekiares (2000).

STUDY	BODY MASS (g)	HINDFOOT (mm)	EAR LENGTH (mm)	TAIL LENGTH (mm)	TOTAL BODY LENGTH (mm)	SKULL LENGTH	NASALS (mm)	MAXILLARY TOOTHROW (mm)
<b><u>BARKO</u></b>								
Mean	34.80	24.00	17.00	78.50	181.50	29.55	12.00	3.95
S.D.	0.42	0.00	1.41	9.19	13.44	0.64	0.14	0.07
n = 2								
<b><u>BEKIARES</u></b>								
Mean	32.45	22.83	18.68	77.10	176.88	28.33	11.33	3.59
S.D.	4.71	1.02	2.07	6.01	8.39	0.86	0.80	0.02
n = 40								
<b><u>STATISTICS</u></b>								
t	0.70	1.60	0.18	0.32	0.75	1.97	1.17	0.05
P	x	x	x	x	x	*	x	x
df	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00
P > 0.05 = x								
P < 0.05 = *								
P < 0.01 = **								
P < 0.001 = ***								

the skull ( $\underline{t} = 1.94$ ,  $\underline{df} = 40$ ,  $\underline{P} < 0.05$ ). It was larger in this study. However, when compared only to other cotton mice captured in Missouri (Bekiares, 2000), there was no difference in skull length ( $\underline{t} = 0.89$ ,  $\underline{df} = 6$ ,  $\underline{P} > 0.25$ ).

### Allozyme Electrophoresis

I verified that GPI-1\* was a diagnostic locus, and identical banding was produced using tissue from liver or toe-clips. The allelic mobility was faster (more cathodal) in cotton mice when compared to the allelic mobility of white-footed mice (Figure 2.5). Three of the four cotton mice identified using Hoffmeister (1977) were identified using this non-lethal diagnostic allozyme marker.

### DISCUSSION

The morphological measurements of the cotton mice in this 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 =  $32.87 \pm 4.03$  SD; HF =  $22.75 \pm 1.00$  SD) and Missouri (BM =  $28.68 \pm 8.82$  SD; HF =  $23.5 \pm 1.00$  SD; Bekiares, 2000.). Feldhamer et al. (1998) reported

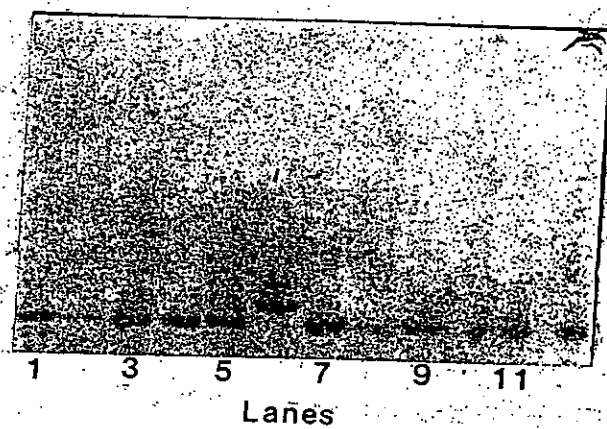


Figure 2.5. Allelic mobility of P. gossypinus (lane 6) and P. leucopus (lanes 1-5 and 7-12) at the GPI-1\* locus.

the average hindfoot length and body mass of Illinois cotton mice were  $22.4 \pm 0.89$  SD mm and  $26.7 \pm 3.10$  SD g, respectively. All adult cotton mice in this study and Feldhamer et al. (1998) adhered to the "general rule" of body mass  $\geq 26$  g or hindfoot length  $\geq 22$  mm as well as the ratios established by Hoffmeister (1977). However, all cotton mice in these studies did not exhibit both of the morphological measurements. Additionally, it appears that the methodology of Hoffmeister (1977) may not be conservative enough because this technique identified individuals as cotton mice that exhibited white-footed mice alleles (see Bekiares, 2000).

My findings suggest that although morphological measurements may indicate a potential cotton mouse, additional identification is needed for positive species identification (i.e., allozyme electrophoresis). Based on morphological measurements alone, I would have misidentified 17 white-footed mice, calling them 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 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 for mark/recapture studies. Toe-clips are also taken in studies that often involve animal movements, species abundance/evenness estimation, and long-term population monitoring. The removal of a toe-clip has minimal affect 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. This 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.

CHAPTER 3 - STATUS OF THE COTTON MOUSE  
(PEROMYSCUS GOSSYPINUS) IN SOUTHERN ILLINOIS

INTRODUCTION

The cotton mouse (Peromyscus gossypinus) is a large woodland mouse that is 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 (Alexander, Johnson, Pope, Pulaski, and Union Counties; Hoffmeister, 1989; see Chapter 2, Figure 2.2).

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 has also been associated with bottomland forests, 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 years specifically to locate them (Feldhamer et al., 1998). The "mystery" of the "disappearance" of the cotton mouse for nearly 90 years is best summarized by Hoffmeister (1989, pg. 215): "What has happened to the P. gossypinus in southern Illinois remains a mystery. Ample search within the last 30 years has been made specifically for these mice. Trapping has been done in habitat that should be suitable for the species but no specimens of P. gossypinus have been found." In 1996, five cotton mice were collected from Horseshoe Lake Conservation Area, Alexander Co., in extreme southwestern Illinois (Feldhamer et al., 1998; Figure 3.1). Other individuals presumed to be cotton mice were trapped and released.

Identification of cotton mice is generally problematic because of their morphological similarity to sympatric species of Peromyscus found in Illinois (Linzey et al., 1976; Hoffmeister, 1989; Laerm and Boone, 1995; Figure 3.2), including the white-footed mouse (P. leucopus) and deer mouse (P. maniculatus). In Illinois, the reported range of the hindfoot length (HF) of adult cotton mice is 22-25 mm (Hoffmeister, 1989). This range slightly overlaps that

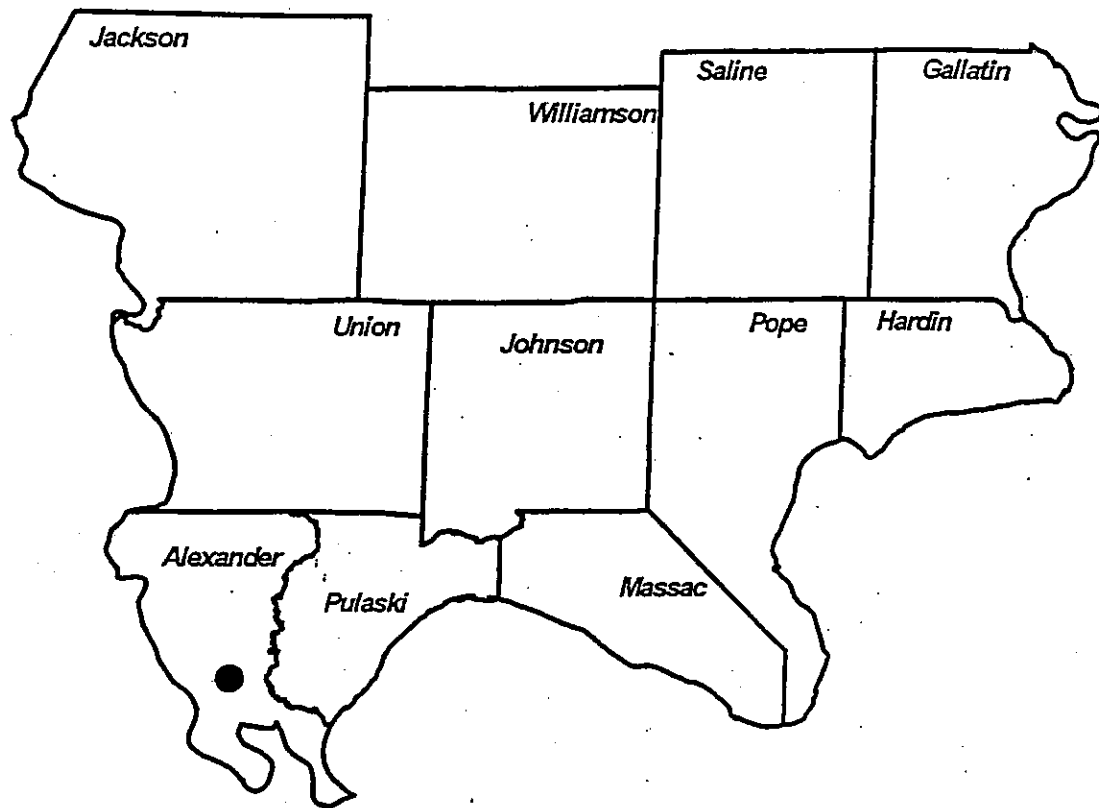
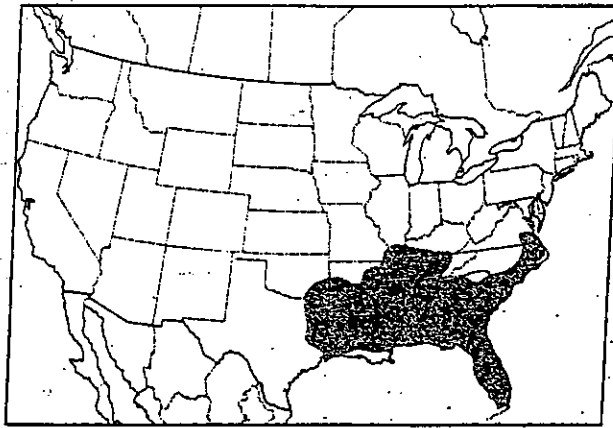
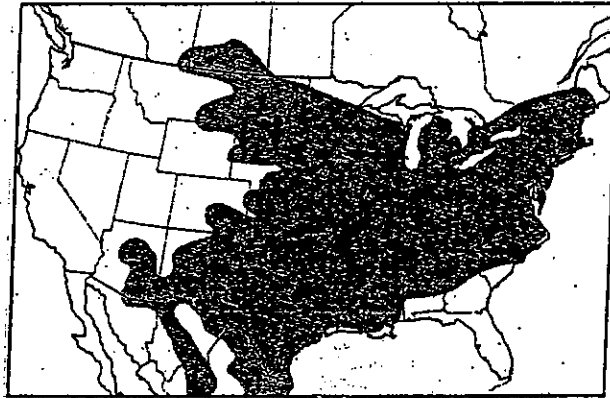


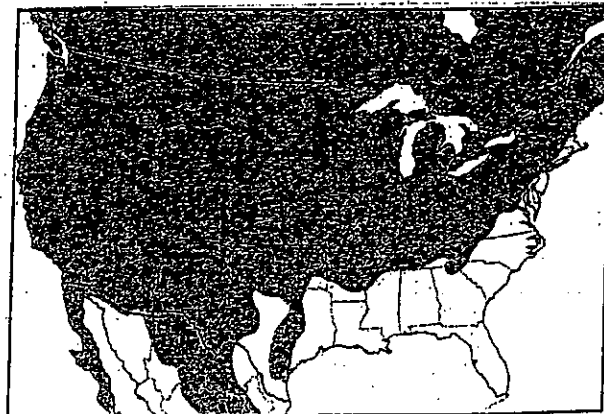
Figure 3.1. Site of presumed cotton mouse captures by Feldhamer et al. (1998) at Horseshoe Lake Conservation Area, Alexander County, Illinois.



Cotton mouse



White-footed mouse



Deer mouse

Figure 3.2. Geographic ranges of sympatric species of Peromyscus in Illinois (Hoffmesiter, 1989).

reported for white-footed mice (HF = 18-22 mm), which slightly overlaps the range reported for deer mice (HF  $\leq$  18mm; Hoffmeister, 1989). Identification is usually based on mensural characteristics (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 hindfoot ranges of cotton mice reported from the northern periphery of the range (Kentucky: Barbour and Davis, 1974; Illinois: Hoffmeister, 1989; Feldhamer et al., 1998, and Missouri: Schwartz and Schwartz, 1981), I established a "general rule" for identifying a potential cotton mouse (hindfoot length  $\geq$  22mm or body mass  $\geq$  26 g). Other studies have reported hindfoot 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 adequately studied congeneric vertebrate groups. Natural hybrids (identification based on morphology) between P. gossypinus and P. leucopus have been reported from areas of sympatry (Howell, 1921; McCarley, 1954; St. Romain, 1974; Lovecky et al., 1979). There is complete interfertility between cotton mice and white-footed

mice (Dice, 1937; 1940). Dice (1940) reported hybrids of these species are completely interfertile when crossed with each other and when backcrossed with their parental species. Cotton mouse x white-footed mouse hybrids are intermediate in size in morphological characters (Dice, 1940; but see Bradshaw, 1968).

It is unknown whether cotton mice have been in southern Illinois since 1909, but 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. In the summer of 1993 flood stages were reached. Flood waters flowed through Horseshoe Lake Conservation Area, and spilled over the dam into Lack Creek. From Lack Creek, the flood waters moved to the Cache River diversion outlet (Bhowmik et al., 1994). Alternatively, small ephemeral cotton mouse populations may occur, but quickly hybridize with the more abundant white-footed mice, or quickly become extirpated.

My objectives were to: 1) determine the current distribution of P. gossypinus in southern Illinois, 2) test the null hypothesis that P. gossypinus is not an ephemeral species in southern Illinois, and 3) test the null hypothesis that P. gossypinus and P. leucopus do not

hybridize in southern Illinois.

## MATERIALS AND METHODS

### Site Selection

Study sites were located using an ArcView Geographic Information System (GIS) v.3.x (Environmental Systems Research Institute, Redlands, New Jersey). Five data layers (palustrine forested wetlands, road system, USGS 7.5 minute quadrangle boundaries, county boundaries, and public land) were combined, from Illinois Natural History Survey data (<http://www.inhs.uiuc.edu>) and Illinois State Geological Survey data (<http://www.isgs.uiuc.edu>), to identify suitable cotton mouse habitat in the 6 southwestern-most counties of Illinois (Alexander, Johnson, Massac, Pope, Pulaski, and Union; Figure 3.3). I considered "suitable habitat" to be any patch that was hardwood bottomland forest, a minimum size of 8 ha (area needed to establish a trapline), and located 100 m from a primary or secondary road (arbitrary value). This 100-m buffer was established because cotton mice are not considered to be "edge" species. To maximize the likelihood of capturing cotton mice, I avoided "edge" habitat typically associated with disturbed areas such as roadsides. Both public and private lands were

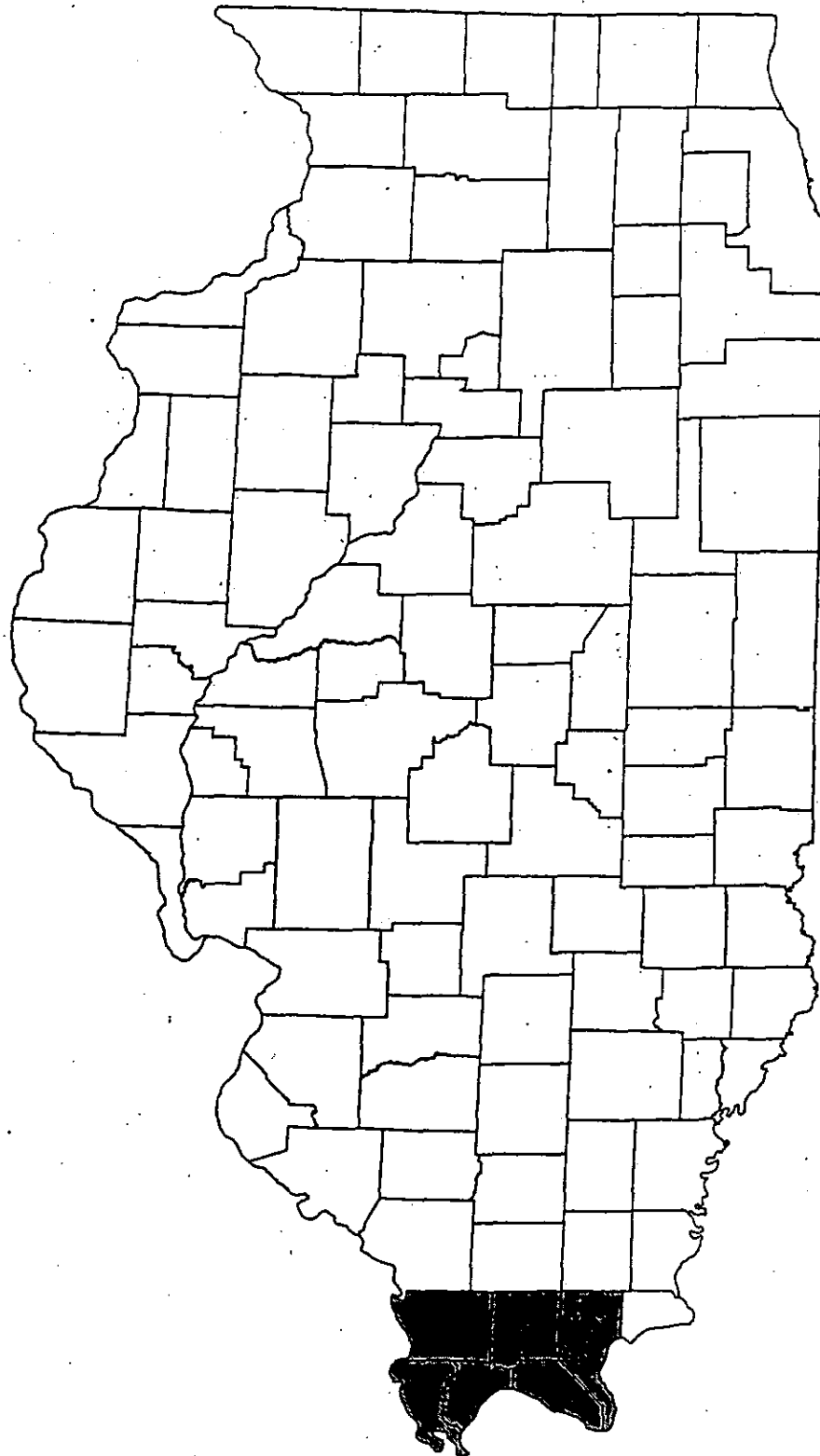


Figure 3.3. Counties of Illinois that comprised the cotton mouse study area.

identifiable (Figure 3.4). Non-deciduous or upland habitats were not surveyed based on the findings of Schmid (1998).

Sixty study sites were chosen systematically in the six counties of interest by dividing the area into USGS 7.5 minute quadrangle boundaries. The total area of bottomland forests was determined for each quadrangle, and 1.75% of each quadrangle was sampled (Appendix 2). This allowed me to spread my sampling effort more evenly across the study area. Fifty-two sites were identified using this method combined with availability of the patches (i.e., public land/landowner permission and water levels). Eight additional sites were sampled, and selection was based on availability. A Magellin Trailblazer XL Global Positioning System (GPS) was used to accurately determine the location of each study site (Appendix 3). The accuracy of the GPS unit was to within 100 m horizontally and 150 m vertically.

#### Small Mammal Trapping

Animals were captured from May 1998 through August 1999 using Sherman live traps (8 x 9 x 23.5 cm; H.B. Sherman Co., Florida) set in a standardized transect. One hundred traps were set at each site, with traps placed 10 m apart. Traps were set in the afternoon near fallen logs, stumps, water



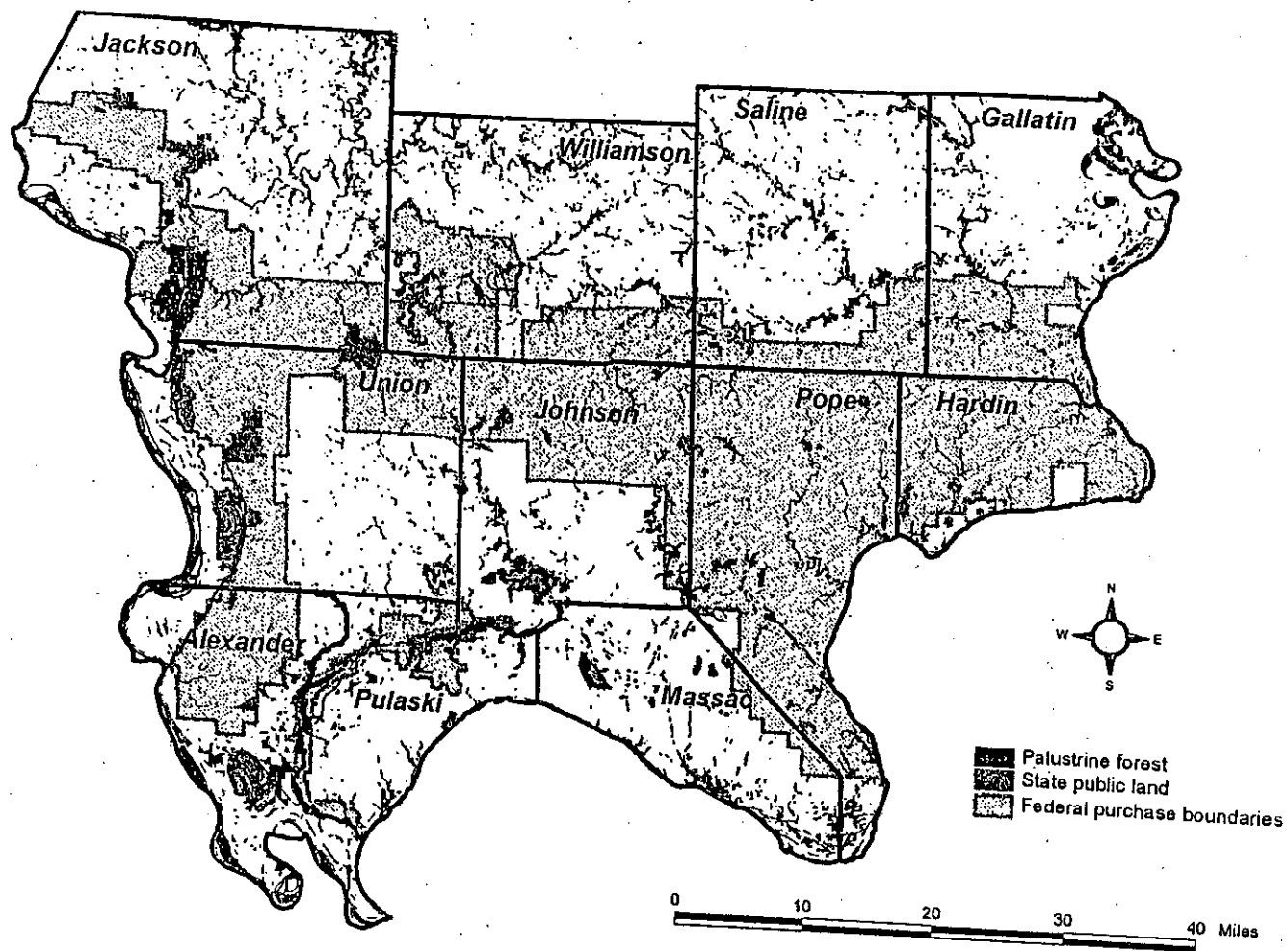


Figure 3.4. Bottomland forest patches located on public and private land in the 6 southwestern-most counties of southern Illinois.

body edges, or tree trunks to increase 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 checked daily between 0600 and 1100 hours. 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 and animal handling followed the methodology suggested by the American Society of Mammalogists (Committee on Acceptable Field Methods, 1987).

#### Tissue Preparation

Toe-clips 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. The tubes were stored on icepacks in a soft-sided cooler until return to the laboratory to prevent denaturing of the proteins (Manlove et al., 1975). Toe-clip samples were then frozen at  $-70^{\circ}\text{C}$  for future allozyme analysis (Hillis et al., 1996).

#### Allozyme Electrophoresis

In a pilot study, Barko et al. (in press) verified glucose-6-phosphate isomerase (GPI-1\*; EC 5.3.1.9) exhibited diagnostic alleles between P. gossypinus and P. leucopus (see Price and Kennedy, 1980 and Robbins et al., 1985). Banding could be produced from toe-clip tissue using cellulose acetate (CA) electrophoresis. This alleviated the use of internal tissue (liver) and the necessity of sacrificing individual animals. I 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).

CA electrophoresis was conducted on potential cotton mice (hindfoot  $\geq 22$  mm or body mass  $\geq 26$  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).

Before conducting allozyme electrophoresis, 80  $\mu$ l of distilled water was added to each sample, tissue samples were homogenized, and homogenates were centrifuged at approximately 10,000 g's for five minutes to separate the supernatant (with enzymes) from cellular debris. Ten  $\mu$ l of the resulting supernatant were placed in an individual loading plate well (Helena Laboratories, Beaumont, TX). A continuous Tris Glycine (pH 8.5) buffer system was used (Herbert and Beaton, 1993; Appendix 1). Gels were electrophoresed at 191 volts for 25 minutes. Following electrophoresis, gels were histochemically stained, scored, dried in an oven, and saved as vouchers (Hebert and Beaton, 1993; Appendix 1).

#### Statistical Procedures

Unpaired t-tests were used to compare morphological measurements (i.e., hindfoot length and body mass) between cotton mice recently captured in Kentucky, Illinois, and Missouri by Feldhamer et al. (1998), Bekiares (2000) and Barko et al. (in press). Because of small morphological characteristics, I was unsure if the mice captured by

Feldhamer et al. (1998) were cotton mice or natural hybrids. Only adult animals, based on pelage coloration and body mass (> 18 g) were used in analyses (Cummings and Vessey, 1994; Nupp and Swihart, 2000) and  $\alpha = 0.05$  (Steel and Torrie, 1980).

## RESULTS

### Peromyscus Captures and Trapping Success

A total of 1309 Peromyscus sp. was captured and toe-clipped during 18,000 trap nights (trap success rate = 7.3%).

### Allozyme Electrophoresis

One-hundred eighteen mice were screened at the diagnostic GPI-1\* locus as potential cotton mice (hindfoot length  $\geq$  22 mm and/or body mass  $\geq$  26 g) and 266 mice were screened at the same locus to verify that they were white-footed mice (random sampling of 25%). One potential cotton mouse was identified as a hybrid (body mass = 22 g), based on a heterozygote GPI-1 marker, and one mouse from the random sampling (hindfoot length = 21 mm; body mass = 18.5 g) exhibited the cotton mouse allele (Figure 3.5). All other screened mice (382 individuals) were identified as

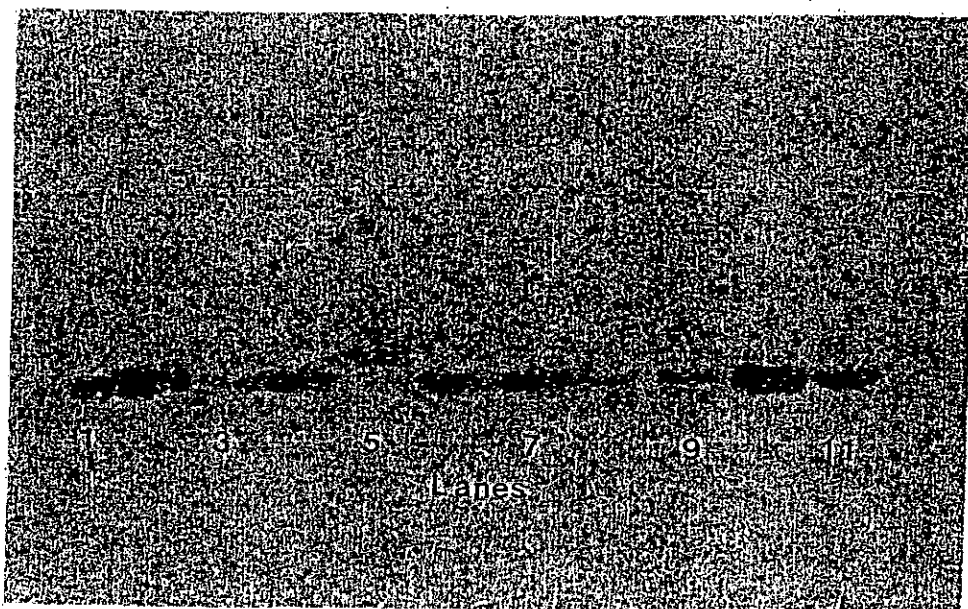


Figure 3.5. Cellulose acetate gel of the cotton mouse (lane 12) identified in Illinois at the GPI-1\* locus. The standard (known cotton mouse liver tissue) is in lane 5. All other lanes contain toe-clip tissue from white-footed mice.

white-footed mice. The remaining mice (925 individuals) were presumed white-footed mice based on morphology and electrophoretic results of the random samples.

#### Comparison of Cotton Mice Collected from MO, IL, AND KY

Cotton mice captured in Illinois by Feldhamer et al. (1998) were generally smaller, based on mean hindfoot length and mean body mass, than cotton mice recently captured in Missouri (Barko et al., in press; Bekiares, 2000) and Kentucky (Bekiares, 2000; Table 3.1). There were no statistically significant differences between the means of hindfoot length and body mass of the Missouri and Kentucky cotton mice (see Chapter 2, Table 2.3).

#### DISCUSSION

The "general rule" for identifying cotton mice (hindfoot length  $\geq$  22 mm or body mass  $\geq$  26 g) did not enable me to accurately identify a cotton mouse in Illinois. One-hundred eighteen mice had one or both of these criteria and none were actually cotton mice based on genetic testing. The individuals with the cotton mouse and hybrid alleles had morphological measurements within the range reported for white-footed mice, and would have been misidentified without

Table 3.1. Morphological measurements of presumptive cotton mice collected by Feldhamer et al. (1998) compared to those collected by Bekiaries (2000) and Barko et al. (in press).

BODY MASS (g)					
Means	S.D.	df	t	n	P
Feldhamer = 26.7	3.10	6	7.46	5	***
Barko = 34.8	0.42			3	
Feldhamer = 26.7	3.10	43	5.67	5	***
Bekiaries = 32.4	4.71			40	

HINDFOOT LENGTH (mm)					
Means	S.D.	df	t	n	P
Feldhamer = 22.4	0.89	6	2.85	5	**
Barko = 24	0			3	
Feldhamer = 22.4	0.89	43	0.89	5	x
Bekiaries = 22.8	1.02			40	

$P > 0.05 = x$

$P < 0.05 = *$

$P < 0.01 = **$

$P < 0.001 = ***$



the use of genetic testing.

I suspect P. gossypinus and P. leucopus hybridize in southern Illinois, when the occasional cotton mouse disperses into the area. The small cotton mouse and hybrid Peromyscus I identified using the GPI-1\* marker could have been back-crossed individuals with P. leucopus. This could help explain the small hindfoot length and body mass of both individuals. Backcrossing often masks morphological differences between the species (McCarley, 1954). One disadvantage of the non-lethal technique used is that only one locus was examined. Because multiple loci were not examined, I had a 50% probability of misidentifying an  $f_1$ -hybrid, that is a back-crossed individual exhibiting the P. leucopus allele at the GPI-1\* locus, but P. gossypinus alleles at other loci. I also was 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, the use of this technique did allow me to identify a hybrid individual and gives me an indication of the conservative or minimum level of hybridization in southern Illinois between P. leucopus and P. gossypinus.

Further evidence for hybridization between P. gossypinus and P. leucopus in southern Illinois is suggested

by the results of Feldhamer et al. (1998). They identified five cotton mice (P. g. 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 identify a presumptive cotton mouse unless both methods established an individual as a cotton mouse. However, no tissue samples were saved for genetic analysis and presumptive cotton mice often fell along the scattergram line of Hoffmeister (1977) separating P. leucopus and P. gossypinus.

In a recent study in Kentucky and Missouri, Bekiaries (2000) tested the methods of Hoffmeister (1977) using genetic analysis (i.e., allozyme electrophoresis). She identified individuals on or near the scattergram line as white-footed mice after genetic testing at several loci, including the diagnostic GPI-1\* locus. This suggests the mice identified by Feldhamer et al. (1998) could be hybrids, based on their line position between cotton mice and white-footed mice, when 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 hindfoot length of specimens captured in Illinois, Missouri, and Kentucky. As noted, the mean measurements of cotton mice collected by Feldhamer et al. (1998) compared to those of adult P. g. megacephalus in Kentucky and Missouri (Bekiares, 2000; Barko et al., in press) were significantly smaller (Table 3.1). However, Feldhamer et al. (1998) reported significant differences between cotton mice and white-footed mice from Horseshoe Lake Conservation Area. Unpaired t-tests revealed no significant differences between the mean hindfoot length and body mass from cotton mice in Missouri and Kentucky (Bekiares, 2000; Barko et al., in press; Table 3.2). These findings agree with Bradshaw (1968), who reported hybrids of cotton mice and white-footed mice had morphological characters intermediate in size.

I speculate that cotton mice are an ephemeral species in southern Illinois and disperse into the area occasionally or only during extreme environmental changes such as the large-scale floods of 1993 and 1994. The few immigrants into Illinois are likely to hybridize with P. leucopus. Studies have shown that although cotton mice

prefer to breed with their own species, they will breed with white-footed mice if there is no 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 mate choice (intraspecific) was exhibited in sympatric populations of cotton mice and white-footed mice. Interfertile offspring are produced between cotton mouse and white-footed mouse matings (Dice, 1968), and natural hybrids (based on morphological and mensural characteristics) have been reported (Howell, 1921; McCarley, 1954; St. Romain, 1974; Lovecky et al., 1979). The probability of encountering another cotton mouse would be low during flood conditions or at the extreme periphery of their range where conspecifics are rare or absent.

McCarley (1963) studied distributional 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, that is, show no preference for either habitat type. However, white-footed mice are mainly found in upland areas when they are sympatric with cotton mice (McCarley, 1963). In areas of allopatry and sympatry, cotton mice are mainly found in

bottomland forests. McCarley (1963) concluded cotton mice prevent white-footed mice from inhabiting bottomland forests in areas of sympatry, and create alltopic distribution patterns. This is consistent with my suggestion that cotton mice are an ephemeral species in southern Illinois. All 1307 individual white-footed mice were captured in bottomland hardwood forests; the only habitat in which trapping was conducted. This suggests 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 forests (Hoffmeister, 1989; Schmid, 1998). Based on habitat distribution alone, white-footed mice in southern Illinois follow a pattern similar to allopatric, not sympatric populations.

I suggest occasional hybridization occurs between cotton mice and white-footed mice in southern Illinois because morphological differences appear to remain between cotton mice and white-footed mice. The small size of cotton mice (all males) captured by Feldhamer et al. (1998) likely was the result of  $f_1$ -hybridization. I most likely captured back-crossed individuals which resembled white-footed mice, but still carried cotton mice alleles. All of the cotton mice identified in Illinois during the past four years have

been in the extreme southwestern portion of the state, located in Alexander and Union Counties (Figure 3.6). The distribution of recently captured cotton mice in Illinois is consistent with vicariance flooding events (Bhowmik et al., 1994). I suggest cotton mice recently re-entered southern Illinois via flood waters from the Mississippi River, at the convergence with the Ohio River at Cairo, Illinois. Individual cotton mice probably dispersed into Illinois from Kentucky, which is the closest population. It is probable that some of these cotton mice bred with available white-footed mice, because of small population size and reduced mate choice. This is a plausible explanation for my 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 cotton mice captured in nearby Missouri and Kentucky.

Intensive genetic screening (i.e., collection of internal tissues to screen many loci) of Peromyscus should be conducted in Illinois, as well as in other areas of sympatry on the edge of either species range, to detect and document hybrids. Small mammal surveys in bottomland

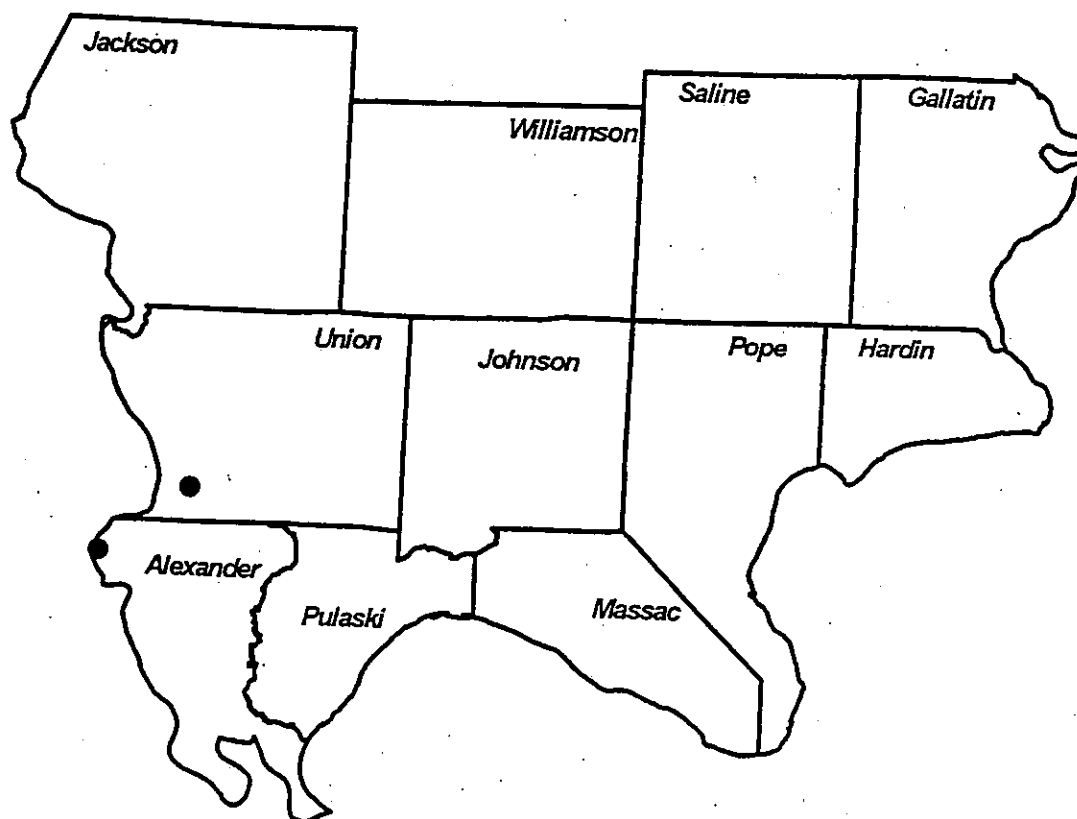


Figure 3.6. Site of cotton mouse (Union County) and introgressed Peromyscus (Alexander County) captured in this study.

hardwood forests, located in the five southernmost counties of Illinois affected by severe flooding should be conducted in the future, especially at the confluence of the Mississippi and Ohio Rivers. This would allow for a better understanding of the community dynamics of a rare species at the periphery of its range, including the extent of congeneric hybridization.



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APPENDICES

Appendix 1. Cellulose acetate electrophoresis buffer and stain recipes for glucose-6-phosphate isomerase (GPI). Recipes follow Hebert and Beaton (1993).

**BUFFER (Tris Glycine (TG), pH 8.5)**

30 gm Trizma base

144 gm Glycine

Make up to 1 liter. Dilute 1:9 (TG:water) for general use.

**STAIN RECIPE (GPI)**

1.0 ml Tris HCl, pH 8.0

1.5 ml NAD (2 mg/ml)

5 drops Fructose-6-phosphate (20 mg/ml)

5 drops MTT (10 mg/ml)

5 drops PMS\* (2 mg/ml)

1  $\mu$ l G6PDH\*

2 ml agar

\* Photosensitive, add immediately before use

**AGAR RECIPE**

1 gm agar

50 ml H<sub>2</sub>O

Appendix 2. Total area of bottomland forest in each USGS 7.5 minute quadrangle boundary laid over the study area (Alexander, Johnson, Massac, Pope, Pulaski, and Union Counties).

Quadrangle Name	Ha of Bottomland Forests	No. of Sites Sampled
Anna	111.08	0
Bandana	28.57	0
Barlow	13.13	0
Bloomfield	566.05	1
Brownfield	643.91	1
Cache	2300.99	5
Cairo	1191.13	3
Cape Girardeau	129.58	0
Cave In Rock	28.55	0
Charleston	91.20	0
Cobden	39.98	0
Creal Springs	206.79	0
Cypress	1408.34	3
Dekoven	81.24	0
Dongola	756.61	2
Eddyville	6.82	0
Glendale	530.92	1
Golconda	52.81	0
Goreville	70.03	0
Herod	113.35	0
Jonesboro	636.71	1
Joppa	429.82	1
Karbers Ridge	85.17	0
Karnak	2043.00	4
Lick Creek	128.08	0
Little Cypress	810.74	2
Makanda	131.23	0
McClure	1409.20	3
Mermet	794.62	2
Metropolis	810.25	2
Mill Creek	273.43	0
Mt. Pleasant	698.37	2
Neelys Landing	274.19	0
Olmstead	224.96	0
Paducah East	718.08	2

## Appendix 2. Continued.

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Quadrangle Name	Ha of Bottomland Forests	No. of Sites Sampled
Paducah Northeast	203.16	0
Paducah West	11.77	0
Pulaski	805.62	2
Reevesville	952.55	2
Repton	85.00	0
Rosiclare	214.13	0
Saline Mines	207.94	0
Shelterville	437.42	1
Smithland	618.71	1
Stonefort	150.45	0
Tamms	1603.43	4
Thebes	904.51	2
Thebes Southwest	138.24	0
Vienna	627.49	1
Waltersburg	315.29	0
Ware	1142.46	2
Wolf Lake	818.42	2
Wyatt	225.83	0

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Appendix 3. Global Positioning Systems (GPS) coordinates for each of the 60 sites sampled. All readings were taken in the NAD 27 datum and are reported in decimal degrees.

Site Code	Latitude	Longitude
S1	37.59	-89.44
S16	37.43	-89.44
H1	37.11	-89.33
H2	37.14	-89.31
H3	37.12	-89.30
H4	37.15	-89.29
UCCA2	37.41	-89.35
UCCA3	37.39	-89.36
LERA	37.57	-88.85
CE	37.50	-88.76
ME1	37.26	-88.86
ME2	37.26	-88.86
ME3	37.27	-88.85
ME4	37.27	-88.85
HEL	37.18	-88.78
HEL2	37.18	-88.78
CLCR1	37.30	-89.41
CLCR2	37.34	-89.38
MFNP	37.14	-88.68
AEP2	37.18	-88.79
UCCA5	37.43	-89.37
UCCA4	37.41	-89.35
SEC8	37.30	-89.02
KARN	37.30	-88.97
UCCA7	37.41	-89.38
UCCA6	37.42	-89.36
PERK1	37.29	-89.08
FORM1	37.35	-88.90
KARN2	37.31	-88.98
KARN3	37.30	-88.98
BOSS	37.36	-88.94
CAVI	37.43	-88.99
HLIS2	37.15	-89.35
HLIS1	37.14	-89.33
BCR1	37.10	-89.25
BCR2	37.10	-89.26
MASS	37.29	-89.51
THEBES	37.21	-89.46
NC1	37.28	-89.12

## Appendix 3. Continued.

Site Code	Latitude	Longitude
NC2	37.28	-89.08
LARUE	37.59	-89.45
UCCAR	37.37	-89.38
ROTH	37.11	-89.30
MCITY	37.11	-89.15
HL5	37.16	-89.29
FTMASS	37.16	-88.70
SMITHLD	37.17	-88.44
WSMITH	37.17	-88.45
FLAT	37.39	-88.75
SIMP	37.47	-88.76
S51	37.12	-88.49
S38	37.23	-89.20
WINTERP	37.58	-89.44
BMLEVEE	37.58	-89.45
N146	37.45	-89.36
TEARS	37.50	-89.36
CNWR1	37.34	-89.07
CNWR2	37.34	-89.07
ROBBS	37.46	-88.71
NSIMP	37.50	-88.76

Appendix 4. Small mammal richness and abundance data from each of the 60 surveyed bottomland forest patches.

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SITE ID	RICHNESS	ABUNDANCE
S1	2	23
S16	1	26
H1	2	52
H2	2	64
H3	2	69
H4	2	87
UCCA2	3	62
UCCA3	3	34
LERA	4	74
CE	1	28
ME1	3	47
ME2	1	44
ME3	1	70
ME4	3	40
HEL	1	25
HEL2	1	21
CLCR1	2	4
CLCR2	2	14
MFNP	3	33
AEP2	2	37
UCCA5	1	3
UCCA4	3	13
SEC8	1	3
KARN	1	13
UCCA7	3	30
UCCA6	3	20
PERK1	2	18
FORM1	3	17
KARN2	2	8
KARN3	1	4
BOSS	1	9
CAVI	1	34
HLIS2	3	21
HLIS1	1	30
BCR1	1	32
BCR2	2	33
MASS	2	21

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## Appendix 4. Continued.

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SITE ID	RICHNESS	ABUNDANCE
THEBES	2	5
NC1	1	5
NC2	2	8
LARUE	3	4
UCCAR	1	10
ROTH	2	25
MCITY	1	27
HL5	2	53
FTMASS	3	13
SMITHD	1	5
WSMITH	1	2
FLAT	1	7
SIMP	1	6
S51	1	1
S38	0	0
WINTER	1	1
BMLEVEE	2	6
N146	1	4
TEARS	0	0
CNWR1	1	11
CNWR2	1	12
ROBBS	2	4
NSIMP	0	0

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Appendix 5. Type, number, and total ha of each palustrine forested habitat patch sampled (n = 54) for small mammals in the 6 southwestern-most counties of Illinois.

Type	No. Sampled	Size (ha)
PFO/SS1C Scrub shrub/forested, broad- leafed deciduous, seasonally flooded	1	7.56
PFO1A Broad-leafed deciduous, temporarily flooded	22	980.09
PFO1AH Broad-leafed deciduous, dyked or impounded	1	161.69
PFO1C Broad-leafed deciduous, seasonally flooded	23	1589.21
PFO1F Broad-leafed deciduous, semi-permanently flooded	1	0.65
PFO6F Deciduous, semi-permanently flooded	5	289.76
PFO6G Deciduous, intermittently exposed	1	81.02

Appendix 6. Percentage of each habitat class located within a 300-m buffer around surveyed bottomland forest patches in the 6 southwestern-most counties of Illinois. Habitat classes include urban/other (U/O), cropland (CROP), grassland (GRASS), conifer forest (CONF), and deciduous forest (DEC).

SITE ID	U/O	CROP	GRASS	CONF	DEC
NSIMP	27.58	3.94	0.00	0.30	68.18
ROBBS	57.57	19.58	14.54	0.00	8.31
CNWR2	66.27	11.45	22.28	0.00	0.00
CNWR1	57.06	11.71	31.23	0.00	0.00
TEARS	12.65	0.00	4.52	0.00	82.83
N146	19.03	31.11	22.06	0.00	27.79
BMLEVEE	85.89	3.30	3.00	0.00	7.81
WINTERP	69.60	0.00	0.00	0.00	30.40
S38	83.99	8.16	7.85	0.00	0.00
S51	90.33	6.65	3.02	0.00	0.00
SIMP	44.74	14.41	11.72	0.00	29.13
FLAT	97.58	0.00	1.21	0.00	1.21
WSMITH	63.36	3.90	1.81	0.00	30.93
SMITHLD	64.24	11.82	14.55	1.21	8.18
FTMASS	71.08	3.92	15.96	0.00	9.04
HL5	97.89	0.00	1.81	0.00	0.30
MCITY	28.88	63.22	5.16	0.00	2.74
ROTH	58.43	35.84	0.00	0.30	5.43
UCCAR	74.03	20.60	0.00	0.00	5.37
LARUE	89.73	3.93	6.34	0.00	0.00
NC2	49.40	46.99	3.61	0.00	0.00
NC1	57.78	19.77	4.79	0.00	17.66
THEBES	70.48	0.00	0.00	0.00	29.52
MASS	82.74	12.20	1.79	0.00	3.27
BCR2	82.23	12.35	0.00	0.00	5.42
BCR1	29.61	31.72	37.76	0.00	0.91
HLIS1	100.00	0.00	0.00	0.00	0.00
HLIS2	87.35	12.35	0.30	0.00	0.00
CAVI	88.66	0.00	2.09	0.00	9.25
BOSS	76.65	6.89	3.89	0.00	12.57
KARN3	100.00	0.00	0.00	0.00	0.00
KARN2	53.41	41.84	4.75	0.00	0.00
FORM1	61.75	8.73	27.41	0.00	2.11
PERK1	72.21	26.28	1.51	0.00	0.00

## Appendix 6. Continued.

SITE ID	U/O	CROP	GRASS	CONF	DEC
UCCA6	98.79	0.30	0.00	0.00	0.91
UCCA7	57.91	35.22	0.00	0.00	6.87
KARN	80.65	19.35	0.00	0.00	0.00
SEC8	100.00	0.00	0.00	0.00	0.00
UCCA4	59.57	28.27	9.73	0.00	2.43
UCCA5	97.89	0.00	0.00	0.00	2.11
AEP2	84.47	6.47	0.65	0.00	8.41
MFNP	59.61	0.00	1.48	0.00	8.91
CLCR2	77.34	9.97	10.27	0.00	2.42
CLCR1	80.90	6.87	0.00	0.00	12.23
HEL2	74.70	13.55	8.44	0.00	3.31
HEL	34.95	3.95	4.57	0.00	56.53
ME4	95.73	2.44	1.83	0.00	0.00
ME3	87.61	12.39	0.00	0.00	0.00
ME2	100.00	0.00	0.00	0.00	0.00
ME1	94.31	0.00	5.69	0.00	0.00
CE	60.18	0.00	0.00	0.60	39.22
LERA	25.07	0.00	0.00	0.00	74.93
UCCA3	58.79	41.21	0.00	0.00	0.00
UCCA2	86.71	0.00	13.29	0.00	0.00
H4	84.89	15.11	0.00	0.00	0.00
H3	90.66	4.82	1.81	0.00	2.71
H2	100.00	0.00	0.00	0.00	0.00
H1	91.87	7.53	0.00	0.00	0.60
S16	88.82	0.00	9.97	0.00	1.21
S1	82.63	2.40	0.00	0.00	14.97

Appendix 7. Results of Shannon's Index and Pielou's J measurement of evenness for the 60 study sites surveyed for small mammals.

SITE	SHANNON'S INDEX (H')	PIELOU'S J	MAX J-value (lnS)
S1	0.17	0.25	0.69
S16	0.00	0.00	0.00
H1	0.12	0.17	0.69
H2	0.10	0.14	0.69
H3	0.14	0.20	0.69
H4	0.32	0.46	0.69
UCCA2	0.40	0.36	1.10
UCCA3	0.28	0.25	1.10
LERA	0.48	0.35	1.39
CE	0.00	0.00	0.00
ME1	0.32	0.29	1.10
ME2	0.00	0.00	0.00
ME3	0.00	0.00	0.00
ME4	0.23	0.21	1.10
HEL	0.00	0.00	0.00
HEL2	0.00	0.00	0.00
CLCR1	0.57	0.83	0.69
CLCR2	0.66	0.96	0.69
MFNP	0.28	0.25	1.10
AEP2	0.20	0.29	0.69
UCCA5	0.00	0.00	0.00
UCCA4	0.68	0.62	1.10
SEC8	0.00	0.00	0.00
KARN	0.00	0.00	0.00
UCCA7	0.63	0.57	1.10
UCCA6	0.69	0.63	1.10
PERK1	0.34	0.49	0.69
FORM1	0.87	0.79	1.10
KARN2	0.36	0.52	0.69
KARN3	0.00	0.00	0.00
BOSS	0.00	0.00	0.00
CAVI	0.00	0.00	0.00
HLIS2	0.39	0.35	1.10
HLIS1	0.00	0.00	0.00
BCR1	0.00	0.00	0.00
BCR2	0.14	0.20	0.69
MASS	0.20	0.29	0.69
THEBES	0.50	0.72	0.69

## Appendix 7. Continued.

SITE	SHANNON'S INDEX (H')	PIELOU'S J	MAX J-value (lnS)
NC1	0.50	0.72	0.69
NC2	0.00	0.00	0.00
LARUE	1.05	0.95	1.10
UCCAR	0.00	0.00	0.00
ROTH	0.52	0.75	1.10
MCITY	0.00	0.00	0.00
HL5	0.10	0.15	0.69
FTMASS	0.80	0.73	1.10
SMITH	0.00	0.00	0.00
WSMITH	0.00	0.00	0.00
FLAT	0.00	0.00	0.00
SIMP	0.00	0.00	0.00
S51	0.00	0.00	0.00
S38	0.00	0.00	0.00
WINTERP	0.00	0.00	0.00
BMLEVEE	0.45	0.65	0.69
N146	0.00	0.00	0.00
TEARS	0.00	0.00	0.00
CNWR1	0.00	0.00	0.00
CNWR2	0.00	0.00	0.00
ROBBS	0.69	1.00	0.69