Discrimination and Hybridization in Peromyscus

Sympatric Peromyscus leucopus and P. maniculatus from Lyon County, Kansas, were tested for their ability to hybridize in natural surroundings from 24 May 1982 through 14 October 1982. Four metal enclosures, 5 ft. x 5 ft. x 3 ft., exposed to ambient conditions at the Ross Natural History Reservation, were used to house separately heterospecific pairs of both species combinations and homospecific pairs for control groups. The control pairs produced three litters during the course of study. P. leucopus female and P. maniculatus male pairs produced no young. One female hybrid was produced from a P. leucopus male and P. maniculatus female pair.

Species discrimination criteria were determined by use of nesting preference. Test mice were given a choice of nesting closer to either homospecifics or heterospecifics of the opposite sex. Control tests consisted of giving test animals a choice of nesting closer to homospecifics of opposite sex or empty nest boxes. P. leucopus males and P. maniculatus females showed no significant difference in nesting preferences. P. leucopus females and P. maniculatus
males did display an ability to discriminate. All control combinations failed to show significant differences in nesting preferences.

Another facet of this research tested for olfactory discrimination. An olfactorium was constructed from a glass terrarium partitioned into three compartments. Each partition was pierced by a tunnel in which a microswitch, sensitive to the weight of a mouse, closed circuits to electro-magnetic pens which left a continuous record on a Kymograph recorder of tunnel passages by test mice. Total time spent by test mice in each end chamber, one previously occupied by homospecifics of opposite sex and one previously occupied by heterospecifics of opposite sex, was recorded. Control tests left one end compartment previously unoccupied, while homospecifics of opposite sex of the test mice occupied the other end chamber. *P. maniculatus* males spent significantly more time investigating *E. leucopus* female odors than homospecific female odors. All others showed no significant differences in their preferences. In control tests, *P. maniculatus* and *P. leucopus* males spent more time in previously unoccupied end chambers than in end chambers previously occupied by conspecific females. Females of both species did not prefer either end chamber significantly. However, results of these tests are inconclusive because of small sample size.

These results suggest that natural hybridization is possible between *P. maniculatus* females and *P. leucopus* males and that reproductive isolation of these sympatric populations is probably behavioral.
DISCRIMINATION AND HYBRIDIZATION
IN PEROMYSCUS

A Thesis
Submitted to
the Division of Biological Sciences
Emporia State University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Daniel E. Haines
December, 1983
Acknowledgment

I would like to use this opportunity to recognize the people who aided in the completion of this paper. I express my gratitude to Dr. Robert Clarke and to Dr. James Mayo for serving on my committee. Especial thanks to Dr. Dwight Spencer for his help and criticism while serving as committee chairman. Finally, I would like to thank my wife, Joyce, for her patience and encouragement during the more trying priorities brought about during the completions of my graduate study.
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In North America, mice of the genus Peromyscus are one of the most widely distributed mammals. Some species and subspecies of Peromyscus can be separated into two forms: (1) long-tailed, forest, brush, or rock dwelling types; and (2) short-tailed, grassland inhabiting forms (King, 1968). Subspecies of two species, Peromyscus leucopus noveboracensis (white-footed deer mice) and Peromyscus maniculatus bairdii (prairie deermice), were used in this research. Both species are widely distributed throughout North America (Blair, 1950). P. leucopus occurs north of Nova Scotia, west to Montana and Arizona, and southward into southern Mexico. P. maniculatus ranges from Mexico north to Yukon in the west and from Hudson Bay to Pennsylvania, the southern Appalachians, central Arkansas, and central Texas in the east (Whitaker, 1980). P. l. noveboracensis and P. m. bairdii ranges include the eastern one half of Kansas (Cockrum 1952; Hall, 1955).

In the Illinoian Biotic Province (Dice, 1943) the eastern deciduous forest gives way to the prairies of the west (Dice, 1922). In eastern Kansas, which is encompassed within this Province, the grassland-deciduous forest ecotone is prevalent. Grasslands occur, for the most part, on the uplands, and forests are found on flood plains and moist hillsides (Dice, 1923). These two habitat types interdigitate allowing eastern woodland species to occur far westward along the wooded streams. Similarly, western species can extend farther eastward along divide areas. Great Plains fauna and eastern deciduous forest fauna meet in eastern Kansas (Cockrum 1952).
P. leucopus inhabits primarily brushy or wooded areas, whereas P. maniculatus prefers grasslands (Svendson, 1964). Cockrum (1952) described the habitat of P. maniculatus in Kansas as being pastures, meadows, fence rows, and, in fact, almost everywhere, except in woodlands. In eastern Kansas, P. leucopus and P. maniculatus are sympatric wherever woodlands meet or extend into prairie habitats.

Both species are similar in external appearance. In areas where P. leucopus and P. maniculatus both occur, individual specimens are sometimes referred to the correct species only with considerable difficulty and identification is often a matter for the expert (Hall and Kelson, 1959). P. leucopus differs from P. maniculatus in being larger, having a longer, more sparsely-haired and less bicolored tail, and larger hind feet (Cockrum, 1952). Differences in body measurements such as total, tail, hind foot, and pinnae lengths are distinct. In Kansas, P. leucopus has total, tail, hind foot, and pinnae ranges from 155-196 mm, 66-89 mm, 21-23 mm, and 15-19 mm respectively. P. maniculatus measurements range from 127-153 mm, 43-72 mm, 16-20 mm, and 13-17 mm respectively (Bee et al, 1981).

Identification has been achieved by numerous methods. With limited success, Moody (1941) used a red blood cell immune agglutination test. More recently, Aquadro and Patton (1980) distinguished between P. leucopus and P. maniculatus by a simple method of detecting variations in their salivary amylase. Choate (1973) found that the only taxonomically useful cranial feature is the breadth of the rostrum.

Ecologic isolation separates two related populations in the same
region when they are restricted to different types of habitat
Blair, (1950). With *P. leucopus* and *P. maniculatus* populations in
Kansas, this does not totally appear to be the case. These species
are often associated where their habitats are adjoined and are sometimes
captured at the same trap sites (Doty, 1973). Blair (1940a) stated
that these two species often meet along the forest edges. McNair
(1931) reported that *P. leucopus* have been caught 0.5 mile from any
wooded area. Home ranges of the two species overlap broadly where
*P. leucopus* occurs around islands of brush and trees in the grassland
(Blair, 1940b). Nicholson (1941) reported that aggregations believed
to be *P. leucopus* and *P. maniculatus* were, on several occasions,
found together in a nest box in a field not far from a wooded area.
The two species may live together in the same nest box during the
winter (Howard, 1949). In Riley County, Kansas, Dice (1923) reported
capturing two *P. maniculatus* in a sumac community. He also collected
three *P. leucopus* in a meadow community. Fitch (1963) found that
*P. maniculatus* chose an artificial forest slightly more often than
an area of artificial prairie. While collecting specimens for this
research, a gravid female *P. maniculatus* was captured during mid-
March in woodland habitat surrounded by cropland. The two species
do not differ in their requirements for food, water, temperature,
or humidity sufficiently to be the basis for habitat differences
(Dice, 1922; Brown, 1964).

Reproductive barriers other than ecological ones must be protecting
the genetic security of *P. leucopus* and *P. maniculatus* populations.
Dice (1933) failed to obtain hybrid offspring from 69 laboratory
matings of *P. l. noveboracensis* and five different subspecies of *P. maniculatus*. He concluded that these two species are never fertile together and stated that no evidence of inter-breeding in nature has been found. Specimens have been captured in the Lyon County, Kansas, area which cannot be positively identified with respect to the various body dimensions as being of either species (Spencer, personal communication). This indicates that hybridization between these sympatric mice may be possible. Part of this research explored this possibility by attempting to obtain hybrids under semi-natural conditions.

Species discrimination is important in maintaining the distinctness of sympatric species in nature. This appears to be the principal isolating mechanism in many cases (Blair, 1953). McCarley (1964) presented evidence of species discrimination in sympatric populations of *P. leucopus* and *P. gossypinus* and suggested that ethological mechanisms maintain species separation. *P. leucopus*, *P. boylii*, and *P. maniculatus* in southern Missouri occur in separate habitats, normally due to behavioral differences among the species (Brown, 1964). This research attempted to determine if behavioral differences, with respect to species discrimination, are present in the sympatric populations of *P. leucopus* and *P. maniculatus* in east-central Kansas. Preferences in nest box selection were used to detect variations in species discrimination.

Various methods may be used by *Peromyscus* for species discrimination. Visual, auditory, olfactory, or combinations of these senses may serve these mice in identifying homospecifics, especially those living in sympatric situations. Most mammalian species have highly developed olfactory processes which are employed in a communicative
context (Westerhaus, 1975). Chemical communication in many mammals
provides individual recognition with respect to species and sex,
(Doty, 1976). Many mammals have poorly developed vision and rely
on odor rather than sight to assess their environment (Parkes and
Bruce, 1961). With the assumption that *P. leucopus* and *P. maniculatus*
in east-central Kansas can recognize conspecifics based on odor alone,
a final facet of this study attempted to eliminate visual and
auditory stimuli in order to ascertain the importance of olfaction
in species discrimination. Moore (1965) found evidence of species
discrimination based on olfaction alone between sympatric *P. maniculatus*
and *P. polionotus*. Methods similar but not identical to Moore's
were used in this research.

The objective of this research was to determine whether
east-central Kansas populations of sympatric *P. leucopus* and *P.*
*maniculatus* could hybridize, and to explore some of the possibilities
that might reduce hybridizing in nature (i.e. species and olfactory
discrimination).
METHODS AND MATERIALS

Live mice were collected using home-made can-type traps similar to those described by Burt (1927). The traps were baited with peanut better and supplied with cotton nesting material. All mice were captured from various locations in the vicinity of Emporia, Lyon County, Kansas. P. maniculatus were collected from traplines set in native tallgrass prairie at least 0.25 mile from any wooded area (Fig. 1). P. leucopus were captured in mature woodlands (Fig. 2). The majority were caught along the Cottonwood River south of Emporia. Trap lines were checked once daily. Trapping started in December, 1981, and continued through September, 1982. Trapping resumed during February, 1983, and lasted through mid-March, 1983.

Mice collected were brought to the laboratory in my garage. Ambient temperatures were dependent on external weather conditions. Photoperiods were natural, with only brief interruptions from artificial lights. Specimens were identified using criteria combined from Bee et al (1981), Hall (1955), and Cockrum (1952). Tail and hind foot measurements were used as the primary criteria for identification. Pelage color and patterns were used to a lesser extent and considered only when combined with tail and hind foot measurements. P. maniculatus were considered identified when tail measurements were less than 64 mm and hind feet were not more than 20 mm in length. P. leucopus with tails longer than 66 mm and hind feet measuring more than 21 mm were considered characteristic specimens for this study.

The animals' ages were also noted. Mice exhibiting a gray color were considered juveniles (Layne, 1968) and were not used. Males
Figure 1. Typical tallgrass prairie habitat from which *P. maniculatus* were collected.

Figure 2. Typical woodland habitat from which *P. leucopus* were collected.
with scrotal testes were considered adults. Adult pelage color; a light brown color, similar in both species, was the only criterion used to determine adult females.

In the laboratory, the animals were sexed and female reproductive conditions were noted. All females were caged alone for 30 days prior to being tested. Relying on information taken from Svihla (1932) on the gestation periods of Peromyscus (P. leucopus being 22-25 days and P. maniculatus being 23-27 days), 30 day periods were considered sufficient to assure that gravid mice were not used. Litters born in the laboratory were discarded and the females were regarded as suitable for testing even before being isolated for 30 days. No attempt was made to identify estrous females before or during the tests. All mice were caged individually in separate cages.

**Interspecific Hybridization**

At the Ross Natural History Reservation, located in northwest Lyon County, Kansas, two 5 ft. X 5 ft. X 3 ft. galvanized sheet metal enclosures exposed to ambient weather conditions were used for hybridization pairings. Each enclosure was partitioned in the middle by galvanized sheet metal to create two five feet square sections in each enclosure (Fig. 3). Sections were numbered one through four from east to west. One quarter inch mesh hardware cloth was placed in the bottom of each section and covered with approximately one inch of soil. To protect the mice from predators, covers were constructed using one inch mesh poultry netting attached to wood frames (Fig. 3). Brush, small logs, flat rocks, cans, and covered cinder blocks were placed in each pen to provide nesting and escape sites (Fig. 4).
Figure 3. Sheet metal enclosure used in hybridizing experiments at Ross Natural History Reservation. Note galvanized sheetmetal partition and predator cover.

Figure 4. Habitat created within each section of sheet metal enclosures. Note nesting and escape sites provided.
Smooth brome (*Bromus inermis*) sod was placed in approximately one half of each pen. Vegetation was allowed to grow but its height was clipped to discourage mice from escaping through the cage tops. Weather-resistant food containers were fashioned from empty plastic milk cartons and supplied with laboratory chow during the study. Water was provided *ad libitum*.

Pair combinations were introduced into the pens on 24 May 1982 and maintained through 14 October 1982. In pen number one, a *P. leucopus* female and *P. maniculatus* male pair was established. A *P. maniculatus* control pair was put into pen number two, while a *P. leucopus* control pair was established in pen number three. Pen number four housed a *P. maniculatus* female and a *P. leucopus* male. Mice used in these hybridization attempts were not allowed to become familiar with each other until introduced into the enclosures. New pairs of the same sex and species combinations were established when one or both mice of the original pair disappeared or died. Some pairs were replaced when offspring were produced.

**Nesting Preference**

To determine nesting preferences, six cages, each divided into three equal compartments, were used. Three cages were divided into three 8 in. X 8 in. X 10 in. chambers. All sides of each compartment were covered with 0.25 inch mesh hardware cloth. Nest boxes, 3 in. X 3 in. X 3.5 in., were constructed from 0.25 inch plywood and placed in each cage. The remaining three cages were of larger proportions, three chambers measuring 10 in. X 11 in. X 13 in., covered with 0.5 in. mesh hardware cloth. Nest boxes, 4 in. X 4 in. X 4.5 in., were made from
0.25 in. plywood for these cages. All nest boxes had two ends, a bottom, and a half front (Fig. 5).

Two nest boxes were fastened in each center compartment. One was in the upper right rear corner and one was in the upper left rear corner. In each end chamber, a nest box was attached adjacent to a nest box in the center chamber (Fig. 5). The nest boxes were separated only by the mesh hardware cloth of the compartment partitions. Mice, separated by the wire partitions, had full visual, auditory, and olfactory contact even while in the nest boxes. The open tops of the nest boxes were covered by the mesh wire of the cages. Covers that could be raised to inspect individual nest boxes were placed on top of the hardware mesh wire of the cages over the nest boxes.

Laboratory chow and water were supplied ad libitum in each chamber. No nesting material was provided except when colder weather warranted it. A small amount of cotton was used in such cases.

The cages were set up in the laboratory and arbitrarily numbered one through six. The compartments in each cage were identified from left to right as (a), (b), and (c). Cage number one was used to test P. leucopus control mice. Cage number two was used to test P. leucopus males, number three to test P. maniculatus males, number four to test P. leucopus females, and number five to test P. maniculatus females. P. maniculatus control mice were tested in cage number six.

One female P. leucopus was randomly placed in either compartment (a) or (c) of cages two and three. A female P. maniculatus was placed in the remaining end chambers of cages two and three. In the same manner, one male P. leucopus was randomly placed in either compartment (a) or (c) of cages four and five. Male P. maniculatus
Figure 5. Diagram of cages used to test *P. leucopus* and *P. maniculatus* for nesting preference. Note location and construction of nest boxes.
were placed in the remaining end compartments of these cages.

In cage number one either a male or female *P. leucopus*, depending on the sex to be tested, was randomly placed in either compartment (a) or (c). The opposite end chamber remained empty as a control measure. The control test animals were always the sex opposite to mice in the end chambers. Cage number six was operated using the same procedures, except *P. maniculatus* was used as a control species.

Mice were tested from 10 May 1982 through 28 October 1982. Two additional *P. leucopus* males and two *P. leucopus* females were tested from 8 March 1983 through 1 April 1983. Test animals were introduced in the center compartment (b) of each cage. No test animals were allowed to become familiar with mice in the end chambers or the equipment prior to testing. All test mice were of the sex opposite that of mice occupying end chambers. Animals were checked once daily during daylight hours. The nest box that test mice chose to nest in during periods of inactivity was recorded. Positive scores were recorded when test mice nested adjacent to a homospecific nest box. Negative marks were recorded when test mice preferred nesting closer to a heterospecific nest box, or an empty nest box in control experiments. Mice were tested for 10 days and none were used for more than one test. New mice replaced the tested mice at the end of 10 days or when new specimens became available. Data were subjected to the Student t-test at the 0.05 level of significance (Ostle, 1963).

**Olfactory Discrimination**

Olfactory discrimination was tested in a manner similar to methods described by Moore (1965). In the laboratory, an olfacto-
fashioned from a 20 in. X 10 in. X 11 in. glass and stainless steel terrarium. Two partitions were constructed from galvanized sheet metal to create three equal compartments within the terrarium (Fig. 6). This allowed for three glass sides and one metal side in each end compartment. The center section had two glass and two metal walls. A 2 in. X 2 in. X 3.5 in. tunnel pierced each partition. Tunnel floors consisted of a treadle suspended over a microswitch sensitive to the weight of a mouse. The switches closed circuits to electro-magnetic pens, which left continuous records, on an electric constant-speed Kymograph recorder, of tunnel passages by test mice. Total time spent in each end compartment by test mice was calculated from these records. The Kymograph recorder operated at a speed of 24 cm/hr.

To prepare the olfactorium before each test, the apparatus was disassembled and cleansed with a soap and water solution. Parts were reassembled in the same position for every test. Laboratory chow, water, and cotton nesting material were provided in each end compartment. Amount and arrangement within the chambers were as identical as possible in each test. Tunnels were blocked to prevent access to the center chamber but mice were allowed access to the tunnels.

To test male mice, a *P. leucopus* female or a *P. maniculatus* female, randomly selected, was placed in a randomly chosen end compartment. A female of the opposite species was put in the remaining end compartment. They were placed in the olfactorium between 1900 and 2100 hours and caged through the night and next day until just prior to testing. End sections were then considered "soiled." After females were removed from end chambers and tunnels opened, a male *P. leucopus*
Figure 6. Diagram of olfactorium used to test for olfactory discrimination between *P. leucopus* and *P. maniculatus*. Note location of tunnels piercing partitions.
or P. maniculatus, depending on which species was to be tested, was introduced in the empty, "clean" center chamber. The Kymograph recorder was turned on and a constant record was kept of tunnel passages throughout the night.

Female mice were tested using the same procedures. Males instead of females were placed in the end chambers to provide olfactory stimuli.

Control tests consisted of randomly placing in an end chamber a homospecific mouse of the sex opposite that of the test animal. The remaining end chamber was left empty and subsequently odorless or clean. Food, water, and cotton nesting material were present in the clean compartment, arranged in the same fashion as the soiled side. Control mice were tested by placing them in the center chamber and opening the tunnels. Constant records of tunnel passages were then recorded on the Kymograph recorder.

No attempts were made to identify estrous conditions of females in end compartments or test females. Test mice had not been exposed to mice used in end chambers or equipment. None of the mice was tested more than once; however, some mice previously tested were used to provide odors in end chambers. Tests did not begin before 2000 hours or later than 2200 hours. All combinations of sex and species were tested.

Each test was divided into 30 minute time intervals. Within each 30 minute interval, total time spent by test mice was ascertained. Data were subjected to the Student t-test at the 0.05 level of significance.
RESULTS

A total of 81 mice was captured for this research (Table 1). All mice trapped were identified as being *P. leucopus* or *P. maniculatus*. No intermediate or suspected hybrid specimens were trapped. Seven *P. maniculatus* and four *P. leucopus* females were gravid when captured and bore offspring in the laboratory.

**Interspecific Hybridization**

Three different pairs were used in *P. maniculatus* control groups in pen number two. The *P. maniculatus* female of the first pair was discovered missing on day 11. The remaining male was removed and a new pair introduced on day 20. After 28 days, four young were produced. On day 62, all mice were removed and the third and final pair was established. On day 91 the male appeared to be missing. No burrow was found. The female had four offspring 30 days after the adult pair had been established. On day 98 both adults were present, but the young were missing. A small rat snake was found in the *P. maniculatus* control pen on day 106, which might explain the disappearance of the litter. On day 120, another litter of five young was found. It had been 19 days since the discovery of the previous litter. On day 134, the young were removed. Adults remained and produced no more offspring throughout the remainder of the study. A total of three litters yielded 13 offspring (Table 2).

Two different *P. leucopus* pairs were used as control groups in pen number three. The first pair produced three young, discovered on day 69. Young and adults were removed and a new pair was established
Table 1: Species, sex, and total number of *Peromyscus* collected during this research.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>Number of mice</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. leucopus</em></td>
<td>♂</td>
<td>25</td>
</tr>
<tr>
<td><em>P. leucopus</em></td>
<td>♀</td>
<td>20</td>
</tr>
<tr>
<td><em>P. maniculatus</em></td>
<td>♂</td>
<td>20</td>
</tr>
<tr>
<td><em>P. maniculatus</em></td>
<td>♀</td>
<td>16</td>
</tr>
<tr>
<td>total</td>
<td></td>
<td>81</td>
</tr>
</tbody>
</table>

Table 2: Reproductive success of homospecific and heterospecific pairings of *P. leucopus* (*P. l.*) and *P. maniculatus* (*P. m.*) in enclosures at Ross Natural History Reservation.

<table>
<thead>
<tr>
<th>Pair combination</th>
<th>No. of pairs</th>
<th>No. of litters produced</th>
<th>Total no. of young produced</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. l.</em> ♀ X <em>P. m.</em> ♂</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. m.</em> ♀ X <em>P. l.</em> ♂</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>P. l.</em> ♀ X <em>P. l.</em> ♂</td>
<td>2</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td><em>P. m.</em> ♀ X <em>P. m.</em> ♂</td>
<td>3</td>
<td>3</td>
<td>13</td>
</tr>
</tbody>
</table>
on day 76. When the second pair had been together 29 days, four young were found. On day 120 the young were missing. Both adults were present. On day 144, the final day of the experiment, five young were discovered. A total of three litters, made up of 12 offspring were produced (Table 2).

Four separate *P. leucopus* female and *P. maniculatus* male pairs were used in the hybridizing attempts in pen number one. On day four the *P. leucopus* female was missing. The *P. maniculatus* male was removed and a new pair was established on day 11. On day 80 the *P. leucopus* female had tunnel ed out of the pen. The pair had been together for 69 days. The burrow was blocked and the pen was repaired to discourage further tunneling. On day 84 the *P. maniculatus* male was removed and the third pair was introduced. The female could not be located on day 93. The female was found on day 106 but the male could not be located. On day 120 the female was removed and the male was still missing. It had been 36 days since the pair was introduced. The fourth and final pair was then established and remained together 23 days until the conclusion of the study. These pairs of this combination did not produce hybrid young (Table 2).

One *P. leucopus* male and *P. maniculatus* female pair was maintained throughout the study period. The mice were present each time the pen was inspected. On day 69, the *P. maniculatus* female had begun to burrow but not toward the outside of the pen. The burrow was blocked and the pen altered to discourage further digging. On day 70, one hybrid offspring was discovered. The adult pair had been together 76 days. On day 84 the hybrid young appeared weaned and was removed on day 93. Since discovery of the hybrid, the adult hetero-
specific pair remained together for 67 days until the end of study. No more young were found. A total of one litter with one hybrid young was produced (Table 2).

Nesting preference

A total of 52 different mice were tested for nest box preference: eight *P. leucopus* males, eight *P. leucopus* females, six *P. maniculatus* males, and six *P. maniculatus* females; and six male *P. leucopus*, six female *P. leucopus*, six male *P. maniculatus*, and six female *P. maniculatus*, which were used in control tests.

Male *P. leucopus* displayed no significant preference to nest closer to conspecific females than to *P. maniculatus* females. Six males were tested during a period lasting from 11 May 1982 through 16 October 1982. Two more males were tested from 8 March 1983 through 29 March 1983. *P. leucopus* males chose to nest adjacent to homospecific females 48 of 80 observations (Table 3).

*P. leucopus* females showed a significant difference by nesting closer to homospecific males 63 of 80 times (Table 3). Six *P. leucopus* females were tested during a period lasting from 13 May 1982 through 18 October 1982. Two more females were tested from 11 March 1983 through 1 April 1983.

*P. maniculatus* males also exhibited a significant difference in nesting preference. *P. maniculatus* males chose to nest closer to homospecific females than to *P. leucopus* females 56 of 60 observations (Table 3). Tests were run during a period lasting from 13 May 1982 through 28 October 1982.
### Table 3: Nesting preference data of *P. leucopus* (P. l.) and *P. maniculatus* (P. m.).

<table>
<thead>
<tr>
<th>Species and sex of test mice</th>
<th>No. tested</th>
<th>Times nested closer to homospecifics</th>
<th>Times nested closer to heterospecifics</th>
<th>Total no. of obs.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. l.</em> ♂</td>
<td>8</td>
<td>48</td>
<td>32</td>
<td>80</td>
</tr>
<tr>
<td><em>P. l.</em> ♀</td>
<td>8</td>
<td>63</td>
<td>17 *</td>
<td>80</td>
</tr>
<tr>
<td><em>P. m.</em> ♂</td>
<td>6</td>
<td>56</td>
<td>4 *</td>
<td>60</td>
</tr>
<tr>
<td><em>P. m.</em> ♀</td>
<td>6</td>
<td>31</td>
<td>29</td>
<td>60</td>
</tr>
</tbody>
</table>

* Significantly different at 0.05 level.
No significant difference was found in the *P. maniculatus* female nesting preference. *P. maniculatus* females chose to nest closer to homospecific males than to *P. leucopus* males 31 of 60 observations (Table 3) and were tested during a period lasting from 10 May 1982 through 4 August 1982.

In *P. leucopus* control experiments, males and females showed no significant differences in their nesting preferences. *P. leucopus* males chose to nest closer to homospecific females than to an empty box 39 of 60 times (Table 4). *P. leucopus* females chose to nest closer to homospecific males than to an empty nest box 25 of 60 observations (Table 4). Males were tested from 2 June 1982 through 3 October 1982 and females were tested from 13 May 1982 through 18 October 1982.

*P. maniculatus* male and female control mice displayed no significant nesting preferences. The males were tested during a period lasting from 14 June 1982 through 23 October 1982 and chose to nest closer to homospecific females than to an empty nest box 35 of 60 times (Table 4). *P. maniculatus* females chose to nest closer to a nest box occupied by homospecific males than to an unoccupied nest box 26 of 60 observations (Table 4) during a period lasting from 2 June 1982 through 6 September 1982.

**Olfactory Discrimination**

Sixteen mice were tested in the olfactorium; two male *P. leucopus*, two female *P. leucopus*, two *P. maniculatus* males, and two *P. maniculatus* females. The same number and combinations of mice were tested in control situations. All experiments were run during a period lasting
Table 4: Control data collected from nesting preference studies of *P. leucopus* (*P. l.*) and *P. maniculatus* (*P. m.*).

<table>
<thead>
<tr>
<th>Species and sex of test mice</th>
<th>No. tested</th>
<th>Times nested closer to homospecifics</th>
<th>Times nested closer to heterospecifics</th>
<th>Total no. of obs.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. l.</em> ♂</td>
<td>6</td>
<td>39</td>
<td>21</td>
<td>60</td>
</tr>
<tr>
<td><em>P. l.</em> ♀</td>
<td>6</td>
<td>25</td>
<td>35</td>
<td>60</td>
</tr>
<tr>
<td><em>P. m.</em> ♂</td>
<td>6</td>
<td>35</td>
<td>25</td>
<td>60</td>
</tr>
<tr>
<td><em>P. m.</em> ♀</td>
<td>6</td>
<td>26</td>
<td>34</td>
<td>60</td>
</tr>
</tbody>
</table>
from 6 May 1982 through 4 October 1982.

No significant difference was found in total time spent in either homospecific or heterospecific female end compartments by male *P. leucopus* (Fig. 7). *P. leucopus* females also showed no significant preference in total time spent in homospecific or heterospecific male end compartments (Fig. 7). However, at the 0.10 level of significance, a significant difference was evident. Mechanical problems allowed for only 6.5 hours of test time in one *P. leucopus* male test.

*P. maniculatus* males displayed a significant difference by choosing to spend more time in end chambers previously occupied by *P. leucopus* females than to end compartments previously occupied by homospecific females (Fig. 7). *P. maniculatus* females showed no significant preference between end compartments soiled by homospecific males or *P. leucopus* males (Fig. 7).

Male *P. leucopus* control mice spent significantly more time in clean end compartments than in end compartments containing homospecific female odors (Fig. 8). *P. leucopus* females did not show significant preferences at the 0.05 level of significance (Fig. 8), however, at the 0.10 level of significance, a significant difference in total time spent in homospecific or heterospecific male end chambers was evident. The females spent more time in end chambers previously occupied by *P. leucopus* males. Mechanical difficulties allowed for only two hours of testing for one *P. leucopus* female control experiment.

*P. maniculatus* males spent significantly more time in clean end compartments as opposed to end chambers previously occupied by homospecific females (Fig. 8). Time spent in end compartments
Figure 7. Total time during olfactory discrimination experiments spent by test mice in end compartments previously occupied by homospecifics or heterospecifics of opposite sex.
26.

Species and sex of test mice

= total time spent by test mice in end compartments previously occupied by homospecifics of opposite sex.

= total time spent by test mice in end compartments previously unoccupied.

Figure 8. Total time during olfactory discrimination experiments spent by control mice in end compartments previously occupied by homospecifics of opposite sex and end compartments previously unoccupied.
previously occupied by homospecific males by control *P. maniculatus* females was not significantly different from time spent in previously unoccupied compartments (Fig. 8).
Mice of the genus *Peromyscus* have been the subjects of numerous speciation studies. Consequently, the taxonomy of these animals below the genus level is complex. Classification schemes give subgeneric, species groups, and species titles in their explanation of the diversity of the genus *Peromyscus* (Osgood, 1909; Hooper and Musser, 1964; Hall and Kelson, 1959). Many species have become as diversified as the variety of habitats within their ranges. This is especially evident in the species *P. maniculatus*, where 35 subspecies have been identified in North America and Mexico (Osgood, 1909). Hall (1955) and Bee et al (1981) reported three subspecies of *P. leucopus* and two subspecies of *P. maniculatus* in Kansas.

*P. l. noborecensis* and *P. m. bairdii*, used in this research, are sympatric in eastern Kansas. Both have been placed in the subgenus *Peromyscus* but belong to different species groups. *P. maniculatus* is classified in the *maniculatus* species group while *P. leucopus* belongs to the *leucopus* species group (Hooper and Musser, 1964).

Dice (1933) concluded that mice in the genus *Peromyscus* are infertile together at the species group level. This research, however, yielded a female hybrid animal from a *P. leucopus* male and a *P. maniculatus* female. This indicates, though taxonomically separable, *maniculatus* and *leucopus* species groups are similar in evolutionary relationships in that the two species groups are not as far apart evolutionarily as once thought.

From these results, it can also be implied, with caution, that specimens captured in east-central Kansas which can not be positively identified as *P. leucopus* or *P. maniculatus* may be hybrids, especially
those caught in sympatric situations. The actual occurrence of hybridization in the wild between these species has not been proven, but one would expect that it occurs rarely.

The hybrid, obtained from my hybridizing experiments, had total, tail, hind foot, and ear measurements of 154 mm, 62 mm, 21 mm, and 14 mm respectively. Accuracy of total length was questionable as the mouse was measured live. It was measured on 3 May 1983 and was at least nine months old.

Although one known hybrid specimen can not be considered typical, the external measurements were intermediate between those of *P. leucopus* and *P. maniculatus*. Body measurements used as identification criteria for this study (Cockrum, 1952; Hall, 1955; Bee et al, 1981) did not identify the hybrid as either species. Total length was intermediate, whereas the tail length was that of an adult *P. maniculatus*. Hind foot measurement was within the *P. leucopus* range. Pinnae lengths measured from the notch to tip were that of *P. maniculatus*.

Pelage color of the hybrid was more or less characteristic of *P. leucopus*. The tail was bicolored though not as sharply as that of a typical *P. maniculatus*. Ears had a definite narrow white margin. Feet and underparts were white with the "ankles" brownish black.

The female hybrid's fertility was not known. It was assumed that the two species are not totally fertile together and the possibility of producing fertile offspring seemed remote. Only one was produced by the *P. maniculatus* female and it was possible that other embryos failed to develop. Assuming that copulation between *P. leucopus* males and *P. maniculatus* females occurs with relative ease and
success, prepartuient physiologic mechanisms could be at least partially responsible for maintaining species distinctness.

The *P. leucopus* female and *P. maniculatus* male crossing attempts were not successful and some difficulty was experienced keeping pairs of this combination together in the same enclosure. During inactive periods when the mice were examined, the two species usually nested together. The possibility that *P. leucopus* females and *P. maniculatus* males were incompatible will be discussed later.

The environment within the metal enclosures was considered favorable for reproduction of these two species. Control pairings of both species were successful in producing several litters during the study. It was assumed that enclosure confinement did not inhibit reproduction and can be eliminated as a factor preventing reproduction by *P. leucopus* female and *P. maniculatus* male pairs.

Behavioral differences would seem to be of more importance than physiological or ecological differences in reproductive isolation of *P. leucopus* and *P. maniculatus* populations in east-central Kansas. Results of the nesting preference experiments indicated some ability of these two species to discriminate between homospecific and heterospecific members of the opposite sex. *P. maniculatus* males exhibited the most striking ability to discriminate between *P. leucopus* and *P. maniculatus* females. The only other mice of these species that showed discriminatory ability were *P. leucopus* females. *P. maniculatus* males apparently favored homospecific females and *P. leucopus* females favored homospecific males. This would help to explain the difficulty in maintaining this combination in the same hybridization enclosure.

The possibility exists that one or both repel each other. Results
of *P. leucopus* female nesting preference control studies indicated that *P. leucopus* females were not attracted by homospecific males. Male *P. maniculatus* were similarly not attracted by homospecific females in control situations. These data imply that preferences for nesting closer to homospecifics of the opposite sex were the result of preferring not to nest closer to heterospecifics of the opposite sex by *P. leucopus* females and *P. maniculatus* males.

This research indicated that *P. leucopus* males and *P. maniculatus* females do not discriminate between homospecifics and heterospecifics of the opposite sex. The possibility of hybridization occurring in wild populations of the two species would be greater for *P. leucopus* male and *P. maniculatus* female crosses than for reciprocal crosses. This was apparent from the hybridization experiment results. The *P. leucopus* male and *P. maniculatus* female pairing was successful whereas reciprocal crosses failed. Data on control nesting preference of *P. leucopus* males and *P. maniculatus* females exhibited no significant differences (Table 4).

Nest boxes may have been preferred without a preference being shown to nest closer to a homospecific of the opposite sex. This problem was suggested by Ford (1968) in a similar study.

Estrous conditions of both test females and females occupying end compartments may have affected results. Lengths of the testing periods, however, were considered sufficient to encompass at least one complete estrous cycle. Estrous cycles of both *P. leucopus* and *P. maniculatus* females were found to be five days (Svendson, 1964), therefore 10 day testing periods were considered sufficient
for test females to experience at least one full estrous cycle
during each test. Females starting test periods not in an estrous
condition were assumed to have gone through a complete cycle within
10 days. Similarly, females in an estrous state at the start of
testing periods would leave and return to an estrous state within
10 days. Males did not tend to alter nesting preference as a response
to possible estrous changes of the end compartment females. Similarly,
females did not tend to alter nesting preference corresponding to
possible estrous changes. This implies that females of *P. leucopus*
and *P. maniculatus* do not prefer to nest closer to homospecific
mates due to their estrous conditions. Therefore, estrous conditions
of female mice used in this study were not considered a major factor
affecting the results obtained.

Olfactory discrimination experiments were inconclusive. Some
difficulties were experienced in obtaining mice for testing; thus
inadequate numbers of animals were tested. Mechanical problems with
the test apparatus rendered some results useless and reduced time in
other tests. Interpretations of mice activities recorded on the
constant speed Kymograph recorder was difficult and subject to error.
All test mice apparently entered the tunnels far enough at times to
close circuits of the microswitches without entering end compartments.
This subjected computations of total time spent in end compartments
to error. Results did show tendencies and some evidence for
discrimination between *P. leucopus* and *P. maniculatus* based on
olfaction.

Moore (1965) stated that initial reactions of mice in olfactory
experiments would have more biological significance than those aspects
of olfactory behavior measured later in the same test. Several possibilities exist and were considered in interpretation of results obtained during the olfactory tests. Test mice would be exploring new, unfamiliar conditions and olfactory stimuli were strongest during the first part of tests. Volatility of odors would decrease olfactory stimulation during later portions of experiments (Moore, 1965). Test mice odors would become more prevalent, tending to mask stimulus odors during later portions of tests. Other behavioral aspects not related to olfactory discrimination, such as eating and grooming, would have occurred after initial test mice explorations of the olfactorium. Finally, as test mice became familiar with their surroundings, they would have tended to claim areas as their own and active investigation of homospecific and heterospecific odors would have decreased.

To eliminate some of these parameters, only the first two hours of each eight hour test were subjected to statistical analysis. Greatest activity was noted during the first two hours by all test mice and it was assumed that the later hours were spent feeding, resting, and carrying out other activities.

Male *P. maniculatus* results implied that some factor or factors, possibly a repellant, were present in female *P. maniculatus* odors. Female *P. maniculatus* used to provide odors in the olfactorium end compartments may not have been in an estrous condition, whereas *P. leucopus* females may have been. Male *P. maniculatus* also favored odorless end compartments in control tests, which further supports a possibility of an avoidance of *P. maniculatus* females. *P. maniculatus* have a mid-ventral sebaceous glandular area, but *P. leucopus* do not
(Doty, 1972a). This may be the source of odors P. maniculatus males seem to avoid. P. maniculatus are known to nest in heterosexual conspecific pairs (Nicholson, 1941). This suggests that some tolerance is present, although in these tests avoidance by P. maniculatus males of unfamiliar conspecific female odors was noted. Spending more time in clean end chambers in control tests may have been cases of avoidance of any mouse odors rather than only P. maniculatus odors. Doty (1972b) found that P. maniculatus males tended to prefer P. leucopus urine odors rather than homospecific urine odors though differences were not significant.

P. maniculatus females did not appear to discriminate between males of either species by odors alone. Apparently, as shown by the nesting preference results, they do not discriminate on bases of sight, sound, or other behavior stimuli. However, Doty (1972b) found that estrous, but not diestrous P. maniculatus females prefer male homospecific urine odors to P. leucopus urine odors. P. leucopus males exhibited the same lack of discriminatory ability as P. maniculatus females and seem to have been actually repelled by female P. leucopus odors in control tests. Avoidance of mouse odors may account for preferences of previously empty or unsoiled end compartments. Apparent lack of olfactory discrimination by P. maniculatus females and P. leucopus males coincide with the lack of discriminatory behavior in nesting preference experiments conducted during this research. Success of the P. leucopus male and P. maniculatus female in producing a hybrid young in the hybridization experiments may be due to an apparent lack of their ability to discriminate between homospecific and heterospecific members of opposite sex.
In olfactory discrimination tests, female *P. leucopus* exhibited no significant difference at the 0.05 level of significance, although, at the 0.10 level, a difference was evident. When given a choice between male *P. maniculatus* or *P. leucopus* odors, *P. leucopus* females chose those of *P. maniculatus* males. These results agree with those of Doty (1973), who found that *P. maniculatus* odors are more strongly preferred by both *P. maniculatus* and *P. leucopus* females.

Doty (1973) stated that estrous *P. leucopus* females spent slightly more time with homospecific male urine odors. This indicates that the *P. leucopus* females used in my olfactory discrimination studies may not have been in an estrous state. Though not significant at the 0.05 level of significance, control *P. leucopus* females preferred homospecific male odors at the 0.10 level. When compared to Doty (1973), my data indicated that the *P. leucopus* females used in my control olfactory discrimination experiments may have been in an estrous condition.

Results of the nesting preference studies have agreed with the results of hybridization attempts. *P. leucopus* males and *P. maniculatus* females did not exhibit an ability to discriminate between homospecifics and heterospecifics; thus they appeared to be compatible, at least in sexual behavior. *P. leucopus* females and *P. maniculatus* males, on the other hand, displayed a marked ability to ascertain species of opposite sex, and difficulty experienced in maintaining this pair combination in the hybridization enclosure may have resulted from them being, at least when they were active, incompatible. It was not known whether *P. leucopus* females and *P. maniculatus* males can overcome physiological barriers that might prevent interbreeding,
but on the basis of this research it was assumed that behavioral differences can not be overcome. Knowing if *P. leucopus* females and *P. maniculatus* males could actually copulate would clarify whether or not these pair combinations are physiologically or ethologically isolated.

*P. leucopus* and *P. maniculatus* populations in east-central Kansas are sympatric and little is known of natural hybridization between them, if any. They must have mechanisms to reduce wasting reproductive energies caused by interbreeding. Hybridizing has little or no benefit, if hybrids are sterile, to either species. Ethological isolating mechanisms are barriers to mating due to incompatibility in behavior (Mayr, 1963). Behavioral mechanisms were shown to be present in *P. leucopus* and *P. maniculatus* used in this study. Since both species have different preferred habitats (Cockrum, 1952; Bee et al, 1981; Hall, 1955; Hall and Kelso, 1959; Osgood, 1909; Svendson, 1964; and Whitaker, 1980), but are not totally ecologically separated (Blair, 1940a, 1940b; Dice, 1923; Doty, 1973; Fitch, 1963; Howard, 1949; McNair, 1931; and Nicholson, 1941), habitat differences are probably due to behavioral preferences in habitat selection. This logic alone is sufficient to prevent most natural hybridizing.

Blair (1940b) stated that prairie deer mice (*P. maniculatus*) never inhabit forest situations and there is a zone of grassland, where the grassland is bordered by forests, into which deer-mice seldom range. This zone varies in width from about 40 to 130 feet. Blair also reported that one adult male wood mouse (*P. leucopus*) had 95 percent of its home range in grassland bordering woodlands. He also presented several other examples of *P. leucopus* with home
ranges extending from forest into grassland situations. Several investigators (Barry and Franq, 1980; Hirth, 1959; and Myton, 1974) reported capturing significantly more male than female *P. leucopus* and attributed this to greater activity by males than by females. This was also noted of *P. leucopus* trapped during this study. Since *P. maniculatus* of either sex are rarely found in typical *P. leucopus* habitat, it could be expected that natural hybrids would be the result of *P. leucopus* male and *P. maniculatus* female crosses. Hybridization experiment results of this research agree with this. It might be assumed that species discriminatory abilities of *P. leucopus* males and *P. maniculatus* females would be further evolved than discriminatory abilities of *P. leucopus* females and *P. maniculatus* males. Data collected during this study indicate otherwise and, in fact, indicate that any natural hybridization would come from *P. leucopus* male and *P. maniculatus* female crosses.

Typical *P. leucopus* woodland habitat is advancing on *P. maniculatus* grassland habitat in some areas (Spencer, personal communication). Islands of woodlands surrounded by grasslands are created and destroyed depending on range management practices in the ecotonal areas of east-central Kansas. The ability of *P. leucopus* to expand their range and cross less than favorable (grassland) habitats would be beneficial to the species. Individuals that occur beyond the usual range of their species often have difficulty in finding homospecific mates and this may be the reason for increased frequency of hybrids near the periphery of the species range (Mayr, 1963). This probably is the case with *P. leucopus* in islands or "fingers" of woodlands extending into, or surrounded by, grasslands. Natural hybridization
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probably does occur between these two species, yet the occurrence of such has escaped documentation.
Sympatric *P. leucopus* and *P. maniculatus* from Lyon County, Kansas, were tested for their ability to hybridize in semi-natural surroundings from 24 May 1982 through 14 October 1982. Four 5 ft. x 5 ft. metal enclosures exposed to ambient conditions at the Ross Natural History Reservation were used to house separately heterospecific pairs of both species combination and homospecific control pairs. The control pairs produced three litters over the course of the study. *P. leucopus* female and *P. maniculatus* male pairs produced no young. One female hybrid was produced from a *P. leucopus* male and *P. maniculatus* female pair.

Species discrimination using nesting preference criteria were determined. Test mice had a choice of nesting closer to either homospecifics or heterospecifics of opposite sex. Control tests consisted of giving test animals a choice of nesting closer to homospecifics of opposite sex or empty nest boxes. *P. leucopus* males and *P. maniculatus* females showed no significant difference in nesting preferences. *P. leucopus* females and *P. maniculatus* males did display an ability to discriminate. All control combinations failed to show significant differences.

Another facet of this research tested for olfactory discrimination. An olfactorium was constructed from a glass terrarium partitioned into three compartments. Each partition was pierced by a tunnel in which a switch closed circuits to electro-magnetic pens which left a continuous record of tunnel passages on a Kymograph recorder. Total time spent by test mice in each end chamber, one previously
occupied by homospecifics of opposite sex, and one previously occupied by heterospecifics of opposite sex, was calculated. Control tests had one end chamber left previously unoccupied, while a homospecific of the sex opposite that of test mice previously occupied the other end chamber. *P. maniculatus* males spent significantly more time investigating *P. leucopus* female odors than homospecific female odors. All others showed no significant differences in their preferences. In control tests, *P. maniculatus* and *P. leucopus* males spent more time in previously unoccupied end chambers than to end compartments previously occupied by homospecific females. Females of both species did not prefer either end chamber significantly. However, results were inconclusive because of small sample size.

These results imply that natural hybridization is possible between *P. maniculatus* females and *P. leucopus* males and that reproductive isolation of these sympatric species involves behavioral isolating mechanisms.
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