HOMOSPECIFIC AND HETEROSPECIFIC ODOR PREFERENCES IN SEXUALLY-NAIVE PEROMYSCUS MANICULATUS BAIRDI AND PEROMYSCUS LEUCOPUS NOVEBORACENSIS

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ABSTRACT

HOMOSPECIFIC AND HETEROSPECIFIC ODOR PREFERENCES IN SEXUALLY-NAIVE PEROMYSCUS MANICULATUS BAIRDI AND PEROMYSCUS LEUCOPUS NOVEBORACENSIS

By

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A review of the small rodent olfactory literature was undertaken, and a hypothesis formulated suggesting that some species of rodents may engage in courtship phenomena conceptually similar to those observed in many species of birds, fish, and amphibia. Olfaction, rather than vision or audition, was suggested as the salient modality used in the mediation of these behaviors.

In Study 1, three female *Peromyscus maniculatus* were tested for preferences of *P. maniculatus* and *P. leucopus* male odors in a two-choice plexiglas olfactorium. Each ovariectomized *S* was tested for four consecutive nights under exogenously-produced estrus, and for four consecutive nights, under diestrus. A preference for homospecific to heterospecific male odor was observed during estrus, but not during diestrus.

Study 2 confirmed these results in 20 naturally-cycling female *P. maniculatus* in 1 1/4 hr. test periods, and found quite reliable modes of responding within test sessions.

Study 3 utilized a 2 x 2 x 2 x 2 analysis of variance design, and examined the preferences of 64 female *P. maniculatus* and *P. leucopus* for homo- and heterospecific male urine odors. Cumulatively-aged urine from *P. maniculatus* males was found to be attractive to *P. maniculatus* females, and possibly to some *P. leucopus* females, when paired with an air alternative. *P. maniculatus* females, but not *P. leucopus* ones, showed a relatively strong tendency to avoid the *P. leucopus* male urine odor. Neither of these effects appeared to be influenced by the estrous state of the *S*.

Using an experimental design similar to that of Study 3, Study 4 tested 64 male *P. maniculatus* and *P. leucopus* for preferences of homospecific and heterospecific estrous and diestrous female urine odors. Male *P. maniculatus* showed a stronger relative attraction to estrous than to diestrous female urine odor (when these were paired with air alternatives), whereas male *P. leucopus* showed just the opposite. However, separate analyses for each species failed to disclose significant effects due to the estrous condition of the females producing the stimulus urine. A strong position preference for one side of the olfactorium was exhibited by the males of this study.

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The *S*s of Studies 3 and 4 crossed less from one side of the olfactorium to the other in the presence of homospecific opposite-sexed urine odor than in the presence of heterospecific urine odor. In these two studies, no systematic relationship was discernable between the age of the cumulative urine build-up and the magnitude of the preference or the number of crosses in the test situation. High intratest reliability coefficients for the percent time spent on a given side were present in Studies 2, 3, and 4.

A comparison of the percent times spent with an opposite-sex urine odor by males and females suggested that female *P. maniculatus* reacted more strongly than male *P. maniculatus* to opposite-sex urine odors in directions that could conceivably result in a higher probability of encounters with conspecific opposite-sexed animals and a lower probability of encounters with heterospecific ones. The evidence for this effect was more equivocal for *P. leucopus*.

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INTRODUCTION

In mammalian species possessing a well-developed sense of smell (macrosmats), many behaviors related to changes in gene frequencies of a given population depend, in part, upon olfactory processes. A number of behavioral patterns listed by King (1967) fall into such a category: breeding patterns, assortative mating, courtship, parental care, social tolerance, ingestion, and agonistic behavior. The present chapter begins with a review of studies demonstrating the close relationship between endocrinology, sex odor perception and preferences, and sexual behavior in a number of male and female rodents. Following this review, an examination of studies evidencing rodent capabilities for species recognition on the basis of smell is made, thereby laying a conceptual basis for the possible influence of olfaction upon behaviors associated with sexual isolation, assortative and selective mating, and population dynamics, in general. After an examination of rodent olfactory "imprinting" studies, an argument for the potentially important role of female rodents in mate selection and sexual behavior is presented, based partly upon knowledge of courtship behavior in a number of non-rodent species, as well as upon sexual and species-specific morphological and behavioral divergence related to olfaction. Subsequent chapters include a series of experiments examining the behaviors of two species of mice from the genus Peromyscus to homospecific and heterospecific odors, in an attempt to begin the delineation of stimuli and species to which this notion potentially applies.

Interactions between Endocrinology, Sex Odor Perception and Preferences, and Sexual Behavior

A close relationship between olfaction, sexual behavior and endocrine functioning has been demonstrated in a number of rodent species. Maternal behavior in mice (Gandelman, Zarrow, Denenberg, & Meyers, 1971) and male mounting behavior in male (Murphy & Schneider, 1970) and early-androgenized female (Doty, Carter, & Clemens, 1971) hamsters are eliminated by the removal of the olfactory bulbs. Olfactory bulbectomy eliminates estrous cycling and reduces the size of the ovaries in one strain of laboratory mice (Whitten, 1956a, 1956b). However, such an operation does not markedly influence cycling in rats (Rosen, Shelesnyak & Zacharias, 1940) or guinea pigs (Donovan & Koprina, 1965). Olfactory bulb removal in the male rat results in a prolongation of response latencies and a reduction in the total number of ejaculations during a test session (Beach, 1942; Heimer & Larsson, 1967). Previous copulatory experience is an important factor in this species since, postoperatively, experienced males show an increase in time to ejaculation, but not in the time to the next intromission, while inexperienced males appear to be deficient in both measures (Bermant & Taylor, 1969).

Perhaps the best known interaction between olfactory and endocrine processes are those demonstrating the influence of male-mouse odors on reproductive physiology. A male mouse's odor can produce estrous synchrony in grouped females, resulting in an increase in the number coming into heat on the third night following its presentation (Whitten, 1956a: Whitten, Bronson, & Greenstein, 1968). This effect has been termed the "Whitten Effect." Bronson (1971) produced this effect in female deermice, Peromyscus maniculatus bairdi, by adding as little as .01 ml. male urine/day for a two-day period to their bedding. Inbred SJL/J Mus musculus females required doses of 1 ml/day and higher, which are quantities not much less than the normal output of a male in a 24 hr. period. The compound producing estrus synchronization is probably either an androgen metabolite or the product of androgen-maintained tissue, since castration eliminates it from the urine and testosterone replacement returns it to castrates of either sex (Bronson & Whitten, 1968).

The smell of "strange" (i.e., non-stud) male mice can prevent ova implantation in recently-inseminated female mice (Bruce, 1959, 1960; Bronson, Eleftheriou, and Garick, 1964) and is commonly termed the "Bruce Effect". Bruce and

Parrott (1960) showed that recently-mated bulbectomized female mice failed to respond to the presence of males by pregnancy block, and Dominic (1964, 1966) found the source of the odor causing the effect to be male urine. Odor from females and castrated males does not effectively block pregnancy, suggesting that the male gonads are involved in producing the blocking agent (Bruce, 1965).

Male odor results in other, more subtle, effects upon both male and female endocrine systems of mice. Odor from strange males increases adrenal weight and decreases adrenal ascorbic acid production in inbred Mus musculus males of the S.A.S.T.O. strain (Archer, 1969; Ropartz, 1966). Ether extracts of coagulating glands of males also cause an increase in adrenal weight (Ropartz, 1967a), although odors from female cages can similarly produce this increase (Ropartz, 1967b). Vandenberg (1967, 1969) has demonstrated that male-mouse odors hasten the sexual maturation (e.g., age at first estrus) of female house mice. Furthermore, bedding obtained from cages of males who lived near estrous females was more effective in hastening sexual maturation than bedding from cages of isolated males. Unfortunately, control odors from males living in a similar situation near diestrous females or near males were not included so determination of the specific factors contributing to the enhancement properties of the stimulus odors cannot be made.

The effects of female odor on the reproductive system of rodents has not received much attention, although the grouping of female mice results in prolonged estrous cycling (Lee and Boot, 1955) and is presumed to result from female odor (Bronson, 1971; Gleason & Reynierse, 1969).

Sexual odor preferences in rats are influenced by hormonal or gonadal states. Sexually-experienced male rats generally prefer the odor of estrous females to that of diestrous ones (Le Magnen, 1952; Carr & Caul, 1962), and although castration eliminates this preference, it does not eliminate the S's discriminative ability, per se (Carr & Caul, 1962; Carr & Pender, 1958; Carr, Solberg, & Pfaffman, 1962). While Le Magnen (1952) and Pranzarone (1969) found that sexually-inexperienced male rats preferred the odor of estrous to diestrous female conspecifics, data from other laboratories suggest sexual experience is necessary to produce the preference in adult males (Carr, Loeb, & Dissinger, 1965; Stern, 1970) and early-androgenized females (Robertson & Whalen, 1970). Pfaff and Pfaffman (1969) present data which possibly support this view, although their design confounded sexual experience and castration, making their findings difficult to interpret. Their data do suggest, however, that urine odors may be sufficient to produce the preference. Among female rats, on the other hand, preference for the normal male odor over castrate male odor requires either the presence of ovarian hormones or previous sexual experience.

Sexually-naive females which are nonreceptive at the time of testing show no consistent preference for either masculine odor. Experienced females consistently prefer normal to castrate male odors, regardless of gonadal state (Carr, Loeb, & Dissinger, 1965).

Homotypical (same sex) odor preferences of rats are reported by Carr, Wylie, and Loeb (1970). Castrated or normal sexually-experienced or inexperienced male rats showed no preference for normal vs. castrated male odors. However, sexually-naive receptive females preferred nonreceptive female odor to receptive female odor. No preference occurred in sexually-experienced receptive, sexually-experienced nonreceptive, and naive-nonreceptive females for these odors.

Immature male rats reared in groups consisting entirely of female littermates (segregated) or of half males and half females (cohabitation) showed no significant preferences for receptive over nonreceptive female odors. However, regardless of rearing group, immature males given 1 mg. testosterone propionate per day for nine days before testing showed a significant preference for the receptive female odor over that from nonreceptive ones. Immature females segregated from males in the early rearing situation showed a significant preference for normal over castrate male odor provided they were made sexually receptive at the time of testing. Immature nonsegregated females sexually-unreceptive at testing showed no such preference.

Furthermore, no consistent preference occurred in either receptive or nonreceptive females living in cohabitation with males.

The foregoing results suggest that the responses of rats to sex odors may vary with age. Adults differ from immature rats in their response to both hetero- and homotypical sex odors. Moreoever, it appears that the experiential and hormonal determinants of their reactions operate differently in the two sexes (Carr, Wylie, & Loeb, 1970).

Olfaction and Species Recognition

Within the order *Rodentia*, there is evidence that species recognition and conspecific mate selection can occur on the basis of olfactory cues alone, although, in most situations, several sense modalities are probably used.

Bowers and Alexander (1967) demonstrated that adult male and female *Mus musculus* could learn a simple Y-maze discrimination between the odors of male *Mus musculus* and male *Peromyscus maniculatus* when reinforced by drinking water. Hahn and Simmel (1968) confirmed these findings, in part, showing that male C57BL/10 mice can learn to discriminate (presumably) odors of C57BL/10 mice from those of *Peromyscus maniculatus*, as well as from C3H *Mus musculus*. Unfortunately the sexes of the stimulus mice were not reported and the presence of visual and auditory cues does not allow a pure olfactory interpretation to be made. This study does suggest, however, that a long-term accumulation of the stimulus animals' fecal matter and urine, as used by Bowers and Alexander (1967), is not a necessary prerequisite for the discrimination. This suggestion, of course, assumes that the auditory and visual cues were minimal in the Hahn and Simmel (1968) study.

Godfrey (1958) presented the odors of two groups (presumably different species or on the verge of speciation) of estrous female bank voles, Clethrionomys, to males in a Y-maze situation, allowing the male to approach either the homo- or heterospecific female odor. Males of a given kind approached the odor of the female of their own kind in preference to an alternative. In some instances, the males would approach females from their own localities in preference to females from other localities. Although hybrids were usually discriminated against, the hybrids did not show a differential preference for hybrid and parental types.

Within the genus Peromyscus, two studies strongly suggest olfaction may be an important factor in influencing conspecific mate selection. Smith (1965) gave allopatric and sympatric male mice (Peromyscus eremicus and Peromyscus californicus) the opportunity to build nests and spend time in one of three connected middle compartments of a linearly-arranged five compartment box. The extreme compartments were separated from the adjacent ones by wire mesh. A homospecific female (state of estrus not noted) was housed in one of the extreme chambers and a heterospecific female in the other. All males, with the exception of three (of eight?)

allopatric *Peromyscus eremicus* spent more time in the compartment next to the homospecific female, an intermediate amount of time in the neutral compartment, and the least amount of time in the compartment next to the heterospecific female. The allopatric males spent less time (relatively speaking) next to the homospecific females and more time next to the heterospecific ones than did the sympatric males, supporting McCarley's (1964) suggestion that isolating mechanisms, in some species, are probably being reinforced in sympatric areas. Constant dark did not significantly change the amount of discrimination (there was a slight nonsignificant enhancement) shown by the sympatric *P. eremicus*, suggesting that olfaction may be an important factor in the discrimination. Smith did not, however, investigate this factor further. It is important to note that all of Smith's *S*s were wild-trapped, so it must be assumed that they were probably not sexually-naive. This may account, in part, for the strong homospecific preferences evidenced by the male *S*s for the females, a quarter or less of whom would be expected to be in estrus. It would have been of interest to have examined the behavior of both estrous and diestrous females to the males of Smith's study.

In a fairly extensive study, Moore (1965) found that both sexes of *Peromyscus maniculatus rufinus* preferred an area of a three-chambered box previously inhabited by homospecifics for an eight-hour period to one previously inhabited for an equal time by Peromyscus polionotus leucocephalus. However, most of Moore's tests used male Ss and boxes previously inhabited by estrous females. The few tests with female Ss used only females in heat, so no indication of preference of diestrous females was determined. Moore's measures were based upon initial 15-minute periods of eight-hour test sessions, defined as "the time from the start of each experiment to and excluding the first 10-minute stay in a single compartment, and not exceeding the first 15 minutes of the experiment (p. 90)." The reasons given by Moore for the analysis of only this initial period were that (a) the initial reactions of the Ss would have more "biological significance" than those aspects of behavior measured later, (b) the level of olfactory stimulation would decrease with time, and (c) a number of behaviors incompatable to the olfactory discrimination were present (e.g., grooming, eating, sleeping). Moore's animals consistently favored one compartment more than the others during periods of inactivity, and, apparently, consistently returned to the same compartment in which they spent their first recorded period of inactivity. Unfortunately, Moore concludes from this observation that " . . . some aspects of behavior of the test animal during an experiment were dependent upon the early reactions of the animal in the experiment (p. 90)." Such a temporal-causal relationship appears, to the present author, to be difficult to support. Moore reports that the mice were most active (in terms of intercompartment crosses) during the early periods of the test session and, as the session continued, gradually settled down in the preferred compartment, thereafter exhibiting only irregular bursts of crossing activity.

Olfaction and Imprinting

The purpose of this section is to examine a number of rodent studies implicating the importance of early odor experiences upon the development of adult behaviors and preferences which, in some instances, may affect sexual and species attachments. While the term imprinting has been liberally used by a number of investigators in this area, the present author believes its use is misleading, since most, if not all, of the phenomena attributed to rodent olfactory imprinting can also be interpreted within a social reinforcement paradigm. However, more data are necessary before the relative roles of imprinting, as typically defined by avian behaviorists, and social reinforcement can be determined.

Imprinting in precocial fowl is assumed by most theorists to be a process which occurs relatively independent of traditional drive-reducing reinforcers such as food. Sign stimuli or other factors are presumed to result in attachment which, in some cases, influences species and/or sexual preferences in adulthood. Rodent interactions in early postnatal life are different from those observed in most precocial fowl in a number of ways. For example, rodent young are usually intimately dependent upon their parent or parents for satisfaction of a number of basic needs (e.g., hunger reduction) for an extended time following birth, as compared to precocial birds. Young rodents are quite helpless, with limited locomotion and telereceptor capabilities (e.g., the eyes and pinnae are usually closed for a number of days after birth). The extended period of contact between the young rodent and his littermates and mother allows the opportunity for the influences of potentially potent reinforcers. Tactual and olfactory inputs may be potent very early, since mystacial vibrassae and body hairs are reported to be present at birth in a number of rodents (e.g., some species of Peromyscus, Layne, 1968) and since electrical recordings from the olfactory bulb have been made as early as three and four days of age in the rat (Salas, Guzman-Flores, & Schapiro, 1969). While the various sensory systems become functional at different ages for different species and strains, the important influence of the timing of their development with various behaviors in the litter situation may influence their reinforcement value and their potential interactions with behaviors and sensory experiences. As a hypothetical example related to odor preferences, let's assume that stimulation of a given sensory system (e.g., the haptic system) is most rewarding during the early stages of sensory functioning, but as the novelty of the stimulation wears off, or habituates, its reward value or reinforcement properties lessen. If a previously neutral olfactory environment (e.g., the litter situation) is present during the period when the stimulation is most rewarding, conditioning occurs and the organism learns to associate the odor with a "pleasurable situation." However, if the odor is presented after the reinforcement value of the tactile stimulation has weakened, the association will not be formed, and a strong preference in later life for a similar odor may not be formed. In addition, it is possible that the frequencies of stimulation from the environment (e.g., amount of licking of the neonate by the mother) may decrease with time, so that both the number and the reinforcing capabilities of sensory inputs may be less at a later than an earlier period of time. This oversimplified hypothetical analysis of the so-called critical period is based upon the assumptions of stimulus novelty as an important functioning reinforcer and differential frequencies of environmental stimulation across time. A number of different hypothetical analyses could be postulated, although the present example is sufficient to illustrate the possible complexities of the litter situation. Similar analyses of developmental-environmental interactions in relation to rodent behaviors have been postulated in similar contexts by a number of authors (e.g., Levine & Mitler, 1971; King, 1968b). Due to the possibility of such complex interactions in the early life of many rodent species, the following discussion minimizes the use of the term "imprinting" in relation to the influences of early odor experiences upon later behaviors.

An important study of male-mouse odor preferences in female mice was done in Italy by Mainardi (1963).[1] Mainardi studied the behavior of estrous *Mus musculus domesticus* females toward male odors of their own subspecies and those of *Mus musculus bactrianus*. "Imprinting" or early influences of odors upon later behaviors was implicated in this study, for female *M. m. domesticus* normally reared by both parents since weaning preferred to be near odors of males of *M. m. domesticus*, whereas females reared only by their mothers, in the absence of adult males, showed no sexual preferences, and were attracted indifferently to *M. m. bactrianus* and *M. m. domesticus* male odors. Sixty-three percent of the females raised by both parents preferred the homosubspecific male odor, 23.33% were indifferent, and 13.33% preferred the *M. m. bactrianus* male odor. Of females reared only by their mothers, 35.9% preferred the *M. m. domesticus* odor, 17.95% were indifferent, and 46.15% preferred the *M. m. bactrianus* odor.

Mainardi, Marsan, and Pasquali (1965) found that female housemice (SWM/Mai strain) reared with non-perfumed

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[1] I would like to thank Linda Coniglio for the translation of this paper.

(normal) parents strongly preferred normal to perfumed males in adulthood, whereas females reared by perfumed parents showed no preference for either normal or perfumed females in adulthood. Males reared with normal parents and males reared with perfumed parents showed no preference for either normal or perfumed females as adults, suggesting that the influence of the early olfactory experience may be more important for the females than for the males of this strain. Unfortunately, due to the artificiality of the odors and of the population used, this study lacks the external validity of Mainardi's 1963 experiment.

Marr and Gardner (1965) tested the effects of early olfactory experience on later social behavior in rats. Mothers and young were rubbed daily from 2 to 30 days postpartum with either (a) Yardley's Red Roses Cologne, (b) Methyl salicylate or, (c) with a control non-odor cloth. Dependent measures were made in adulthood and included a Y-maze choice test for stimulus rats rubbed with the different odors, a sexual-responsiveness test to a normal-odor rat, and a study of open field "emotionality" of offspring of parents formerly reared in one or the other of the three olfactory-treatment groups. The cologne group was found to prefer cologne rats, to be less responsive sexually to normals, and to rear young that were more emotional than those of normal parents. *Ss* reared with litter mates and mothers rubbed with toxic methyl salicylate appeared to be "socially anosmic." While this study helps to establish the importance of early olfactory experience upon later social behavior of laboratory rats, the nonbiological character of the odors makes the interpretation of the results difficult and sharply limits their generality. Furthermore, a careful analysis of possible sex differences (assuming both sexes were tested) was not made.

In a study similar to that cited above, Carter and Marr (1970) found that "imprinting," i.e., early exposure, to ethyl benzoate and acetophenone by guinea pigs influences their social preferences in later life, and that animals exposed to an experimental odor from 1 to 6 days of age showed a greater responsiveness to that odor at a later age than did _S_s exposed from 7 to 12, 10 to 15, or 16 to 22 days of age. Again, however, sex differences were not carefully examined and artificial odors were used.

In a rather complex study, Carter (1969) found sex differences in the effects of early rearing odors upon later odor-preference tests in the guinea pig. Males reared with normal odors showed an aversion to acetophenone when tested at 25 days of age, but this aversion was not found when males were tested at 45 or 65 days of age. Normally-reared females, however, showed an aversion to acetophenone at all three test ages. Unfortunately, due to the nonbiological character of the experimental odors, it is difficult to determine a discrete, plausible explanation for this sex difference. Carter suggests differential sexual maturation factors may be involved, but does not provide data to support this explanation.

Sex differences related to species preferences were also observed in cross-fostered pygmy mice (Baiomys taylori alter) raised with Mus musculus (C57BL/cdj) dams (Quadagno and Banks, 1970). Cross-fostered Baiomys females spent significantly less time than control Baiomys adjacent to a caged-off conspecific, whereas males did not. However, cross-fostered Mus males and females spent significantly less time than controls adjacent to a caged-off conspecific, and did not differ significantly from each other on this measure. Unfortunately, auditory and visual cues were also presumably present which may account for some of these effects, even though the situation was designed in a manner to result in an early encounter of the odor by the S in the modified Y-maze. These authors found that, in general, social behaviors such as allogrooming, approach, avoidance, and fighting were markedly effected by the cross-fostering experience. For example, cross-fostered Mus females, when paired with Mus males and females, showed significantly more avoidance behavior and had fewer encounters than did controls. On the other hand, when paired with Baiomys males and females, the cross-fostered Mus females allogroomed more, approached more, and spent more time in physical contact with the other animal than controls. In male *Mus*, cross fostering resulted in widening the scope of sexual responsiveness to *Baiomys* males and females (cross-species mounting attempts were observed), while actually enhancing conspecific sexual behavior. This study, along with several aforementioned ones, suggests that important sex differences are present in a number of species which should be carefully examined in any experiment related to the effects of early odor experience upon later odor preferences and behaviors.

Some Considerations of the Neglect of Female Rodent Behavior and a Call for the Liberation of the Female Rodent

As partially evidenced by the aforementioned studies, the sexual behavior of the male rodent has been given a more prominent role in the literature than that of the female. During the decade from 1960 to 1969, inclusive, 74% of all studies of rat sexual behavior published in the *Journal of Comparative and Physiological Psychology* (n=43) examined the behaviors of only males, 21% the behaviors of only females, and 5% the behaviors of both males and females. Even these figures, however, are conservative in relation to the proponderance of males used in nearly all other types of mammalian behavioral work. The present discussion examines three of many salient interacting factors contributing to this outcome.

First, at least in psychology, rodent behavior has traditionally been studied with the idea of ultimately generalizing to other members of the animal kingdom, most particularly Homo sapiens. This is evidenced by the attempts of many learning theorists to create monolithic theories of behavior. The financial institutions supporting many investigations want generalizations that can be used for pilots, spacemen, underwater divers, and other men in modern male-oriented American society.[2] Second, man as a predominantly visual creature, tends to define his science of behavior in visual terms. When we look at the sexual encounters between rodents, the male appears visually to be more aggressive than the female and, thus, the instigator of contact between the two sexes. Third, and perhaps the more salient of the three, is the nature of behavioral scientific enterprise. It is generally agreed that the end result of scientific inquiry is understanding, the degree of which is usually demonstrated by the precision of prediction and control. The estrous cycle of female rodents drastically alters variables typically used (or which typically confound variables used) in most behavioral studies (e.g., activity,

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[2] An example of these influences in human psychophysical research is clearly provided by Clark in two reviews of human vestibular work, where no studies are presented examining any aspect of vestibular functioning in females (Clark, 1967, 1970).

excretion, emotionality, taste, smell, metabolic regulatory mechanisms, ingestion, sleep, etc.) and is generally a nuisance for many behaviorists. Male subjects, on the other hand, don't present much difficulty in relation to these factors, and can be grouped with either sex without becoming pregnant, pseudopregnant, or just plain poignant. A statement by Godfrey (1958) typifies this point of view "It may well be that females are more discriminating than males in their choice of a mate, but male mammals move about more when sexually aroused, and their choice is therefore easier to score (p. 50)." An examination of the important role of female behavior in influencing evolutionary processes will be presented in the next section, followed by possible implications for the analysis of rodent populations.

Female Behavior and Implications for Sexual Isolation in Non-Rodent Species

Although the behavior of female rodents has been generally ignored by behavioral scientists, at least in the past, this cannot be said for the females of a number of other groups. Beach (1970) found that female dogs showed strong individual preferences for some males but not for others in mating preference tests. Beach (1970, p. 147) concluded that

> (1) anestrous females exhibit varying degrees of social affinity for different males with whom they have been reared, but

(2) under the influence of hormonal conditions associated with estrus these social preferences are obliterated or overridden and an entirely new pattern of heterosexual affinities and aversions emerges.

Beach and Merari (1970) found marked changes in the behaviors of female dogs directed toward visiting males as a function of the female's gonadal state, in tests where the females were confined to wire cages. Following estrogen treatment, some of the females showed sexual presentation responses when males approached their cages. The bitches positioned themselves directly in front of the males so that their perineal areas were immediately proximal to the nose of the male. The females either held their tails vertically or to the side, achieving maximal exposure of the vulva. Some females were observed to move backward and literally force the vulva against the male's snout.

Ethologists have extensively examined female behavior in relation to evolutionary processes in many species of birds and fishes--animals whose courtship and mating patterns are most amenable to visual (and, in some instances, auditory) analysis. It is the contention of the present author that a number of generalizations from ethological analyses also pertain to some rodent species (particularly those forming pair-bonds), although the dominant sensory system of rodents may be olfaction in many cases. An attempt will be made, in the following sections, to examine some of these generalizations in relation to speciation and sexual isolation and to present evidence suggesting these factors are probably operative within the order <u>Rodentia</u>, although, at least in some cases, in terms of olfactory elements rather than visual ones.

In a review of a large number of ethological studies, Hinde (1959) suggests that the evidence for the reduction of interspecific mating via differences in sexual behavior falls into the following categories: (a) rarity of hybridization in nature; and (b) specific differences in courtship postures, colors, or voice between sympatric species. In terms of the latter category, Hinde makes the following generalizations from studies of birds, fish, amphibia, and reptiles:

> (a) Divergence is most marked in those characteristics which are important in pair formation. Thus, in birds with long "engagement periods," early courtship displays differ more between species than those displays occurring immediately before copulation;
>
> (b) Divergence is most marked in areas such as the overlap zone of partially sympatric species, where mating is otherwise possible;
>
> (c) Species characteristics related to sexual isolation often disappear on islands where no closely-related species occur; and
>
> (d) Divergence is more marked in males than in females.

Hinde (1959, p. 90) states:

> In nearly all families of birds the males have diverged in colours and courtship displays more than the females. This is to be expected on Dobzhansky's view of selection for reproductive isolation; since gamete loss is more serious for females than for males, it is primarily female preference that will be selected for.[3]

The role of the female in courtship and pair formation is well illustrated in two generalized statements from ethological analyses of bird and fish behavior. Hinde (1959, p. 91) states:

> In song birds which pair on their territories, the female is usually first attracted by the song or appearance of the male. When the male sees another bird of the same species approaching, he behaves aggressively. If the intruder is an unreceptive female or another male, it either flees or threatens back, and a fight develops. If, however, it is a receptive female, she stays around, perhaps showing some hostility but usually in a 'submissive posture,' which seems to reduce the male's aggressiveness. Gradually the male's behaviour towards the female becomes less aggressive, and pair formation is accomplished (Howard, 1929; Tinbergen, 1939; Lack, 1939 and many later writers).

Bastock (1967, p. 26) cites features of courtship in territorial fish:

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[3] Only a few groups of birds have more brightly colored females than males (e.g., the Red-necked Phalarope of artic and subarctic areas (Tinbergen, 1958)). It is important to note that the degree of dimorphism varies geographically in many species of birds (Mayr, 1964). The loss of sexual dimorphism through feminization of the male plumage seems to occur only in localities where no other similar species exist, i.e., where a highly specific male plumage is not needed as a biological isolating mechanism between two species. In the species listed by Mayr (1964, p. 49), it is suggested that the males lost their bright plumage secondarily. A discussion of a number of species where reversed sex characteristics are found is presented in Wynne-Edwards (1962, p. 237+).

> Where there is territorial behaviour, courtship has a typical opening phase. A female seeks out a male on his territory attracted by his bright colors and conspicuous behaviour (which also warn off other males). She is challenged (or attacked) and responds with a signal indicating her sex and willingness to mate. Occasionally she fights back, but usually she signals too. Signals may resemble appeasement postures adopted by defeated rivals or, occasionally, they are activities performed exclusively by the female before mating. For example, the female char Salmo alpinus digs a nest in an exaggerated fashion within the male's territory (84). In addition, females often lack the bright nuptial colours of the males. Both signals and appearance tend to check the male's hostility so that it is gradually replaced by sexual behaviour; nevertheless attacks are common in early courtship.

In a number of species, the establishment of a territory and the construction of a nest often precedes, or is a component of, the sexual broadcast stimulus of a species (Schein & Hale, 1965). For example, female pike ready to mate tend to prefer males whose territories include areas of dense, short vegetation which are ideal for rearing young (Fabricus and Gustafson, 1958). Emlen (1957) suggests that female Whydah and Bishop birds (Euplectes) select their mates on the basis of the total mating situation, including the nest frame and habitat, as well as the resident male, whose flight displays induce the female to remain near him. Schein & Hale (1965) suggest that a similar situation occurs in such diverse mammals as Uganda Kob (Adenota kob thomasi) and fur seals, wherein males establish and defend individual breeding territories, and then wait for the females to make their selection of a mate.

Female Behavior and Implications for Sexual Isolation in Rodent Forms with Emphasis upon Behavioral and Morphological Divergence

As suggested in the previous section, the present author contends that the generalized evolutionary statements made by Hinde (1959) apply to a number of rodent species, as well as to those groups from which the generalizations were made. There is, of course, wide-spread and generally-accepted evidence for the probable occurrence of some of these processes in *Rodentia*. For example, it has been found that the *Peromyscus polionotus leucocephalus* population on Santa Rosa Island off the Florida coast differs in a number of features of pelage color and is partially infertile with mainland populations of the same species (Blair, 1943; Sumner, 1930). It appears, to the present author, however, that at least two of Hinde's (1959) generalizations have been overlooked, for the most part, in *Rodentia*: (a) that divergence is most marked in those characteristics which are important in early pair-formation; and (b) that divergence is more marked in males than in females. This section presents evidence suggesting that this shortsightedness stems from the possibility that divergence, in a number of instances, occurs via morphological and behavioral olfactory mechanisms and processes involved in the complex interplay of species recognition, mate selection, pair-formation, and territorial defense.

There appears to be substantial evidence that males of a number of rodent groups possess highly differentiated scent glands, mark territories, and spend time in agonistic behaviors with like-sexed individuals--structures and behaviors which, for the most part, are not as markedly present in the females (Ewer, 1968; Eisenberg, 1963; Wynne-Edwards, 1962). The present author suggests that sexual dimorphism in the olfactory structures and behaviors of rodents may be analogous to the visual sexual dimorphism evidenced in many birds and fishes. As a general rule, it might be expected that male rodents are highly odoriferous, just as male birds are highly colorful, and that female rodents are cryptically-odored, just as female birds are cryptically-colored.[4] Other generalizations may be feasible, but are not necessary for the present analysis. Since most laboratory studies of sexual behavior focus upon the consummatory, rather than the appetitive components of the total sexual sequence (as extrapolated from the persent theoretical analysis), little scientific information is available about the cues used in the early attraction and

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[4] As with any generalization, exceptions occur. Diverse functions are most probably served by marking and urine deposition (Ewer, 1968). With full realization of these functions, the present discussion will focus primarily on factors which may be related to sexual isolation (e.g., avoidance or attraction of hetero- and homospecific odors, respectively, and early appetitive components of courtship behavior.)

initiation of sexual interaction (a modern S-R conceptualization of the appetitive-consummatory gradient is presented in Denny and Ratner, 1970). Thus, Hinde's (1959) suggestion that divergence is greatest for those characters which are important in <u>early</u> pair-formation has been, for the most part, overlooked in most sexual studies, and the possibly erroneous belief that the male initiates the sexual encounter has been propogated. This may be essentially disregarding the early appetitive components of the entire sexual sequence and labeling a number of the consummatory components as appetitive. Bermant (1967) states, in relation to copulatory behavior in rats: "In the copulatory behavior of the rat, the male usually appears to take the initiative. It is therefore convenient to organize the behavioral description around the responses of the male (p. 54)." Such an analysis, while convenient for the study of copulation, may be misleading when extrapolations are made to wild population dynamics.

Two quotations from Wynne-Edwards (1962) vividly demonstrate the paradox inherent in the male-oriented analyses of olfaction in rodent sexual and marking activities. Wynne-Edwards (1962, p. 108) states:

> Though exceptions are common, the tendency of predominance of the male sex in dispersionary activities shows up in the mammals almost or quite as strongly as in the birds. Much of the routine trail-marking falls to the males, and this may account for the apparently uneven sex-ratios not infrequently found by people trapping small mammals.

But Wynne-Edwards (1962, p. 105) also states, in regards to a small insectivore:

> Pearson (1946) has provided a most valuable account of the scent glands of the American short-tailed shrew (Blarina brevicauda). In addition to the pair of glands on the flanks possessed by most of all shrews (Soricidae), there is also a third gland on the midline of the abdomen; the last is composed of greatly enlarged sebaceous glands, whereas in the lateral glands, these are mixed with enlarged sweat glands. The glands are better developed in males than in females, and tend to be the largest in males in breeding condition, and smallest in females which are oestrous, pregnant, or lactating. The odour is offensive, and it is generally stated that in consequence few carnivorous mammals will eat shrews, though they may kill them; hawks and owls, however, take them very freely. As Pearson says, 'No one has explained satisfactorily why shrews possess scent glands . . . If protection from predators is the function . . ., it is surprising that pregnant and nursing females, the most valuable segment of the population, have the weakest scent glands and are left relatively unprotected. It has also been suggested that since shrews have very poor vision, the pairs locate each other during the breeding season by the odour produced in the scent glands, but this seems doubtful, at least in Blarina, in view of the fact that the scent glands are reduced in oestrous females at the very time . . . they should be best developed.'

While these seemingly contradictory observations can possibly be explained in terms of the present author's contentions, the hypothesized role of olfactorily-mediated female rodent behavior in relation to evolutionary processes has, at present, little empirical support. However, there are a number of suggestive studies, falling roughly into three categories: (a) odor "imprinting" studies evidencing differential sex effects; (b) endocrinological studies of female mice in relation to male-mouse odors; and (c) behavioral and morphological studies related to specialized odor producing glands. Since (a) and (b) have been previously reviewed in this paper, only (c) will be examined at this time.

Behavioral and Morphological Studies Related to Specialized Odor-Producing Glands

The role of various types of skin glands in mammalian communication is well documented and reviewed adequately elsewhere (e.g., Mykytowycz, 1970; Ralls, 1971). Sebaceous glands, for example, are found in a variety of body areas in animals from nearly every mammalian order. In general, the size of the sebaceous glands appear to vary with the breeding season, are larger in males than in females, and are under the direct hormonal control of androgenic compounds (Mykytowycz, 1970). Sebaceous proliferation is inhibited by estrogen alone (Hooker & Pfeiffer, 1943; Kupperman, 1944) and possibly in combination with progesterone (Doty & Kart, 1972) in several rodent species. While the exact functions of the sebaceous glands are just beginning to be delineated, it appears they are somehow related to home range or territorial marking, and probably influence the dispersionary dynamics of the population. Bronson and Caroom (1971) have demonstrated that the preputial gland (a large sebaceous gland emptying into the prepuce of the male house mouse) contains a substance which is highly attractive to female conspecifies and which can be deposited during urination. These investigators found that homogenates of the preputial glands were preferred to pooled urine from intact males collected externally, to pooled urine collected from the bladder of intact males, or to an empty container. Recent work has implicated the preputial gland secretion in the elicitation of aggression among male house mice (McKinney & Christian, 1970; Mugford & Nowell, 1970), suggesting that the odor may work in a manner analogous to the visual nuptial displays of many birds--namely, it attracts females and repels males. There is evidence that the elicitation of aggression is not solely dependent upon the preputial secretions, since urine from preputialectomized females given androgen can also elicit the aggression (Mugford & Nowell , 1971b).

Some rodents (e.g., the Mongolian gerbil, Meriones unguiculatus) exhibit discrete marking behaviors related to the deposition of sebum from sebaceous glands. Thiessen, Friend, and Lindzey (1968) found that both male and female gerbils rub a ventral sebaceous gland over low-lying objects, leaving a sebum that is oily to the touch and musky in smell to humans. This response is quite discrete, involving a rapid approach to an object, sniffing it, mounting it, and then pressing the sebaceous area against its surface and dismounting forward. Females mark about half as frequently as males, and castration of the male eliminates the marking behavior, whereas testosterone replacement restores it. Blum (personal communication) tested 18 male and 18 female deermice (*Peromyscus maniculatus bairdi*), who possess a gland similar to that of the gerbil's (Doty & Kart, 1972), in an arena containing a number of small protruding pegs. Two of the males, and none of the females, exhibited a clear-cut ventral marking response. The fact that Blum's mice were housed in rather crowded conditions (15 to 18 mice per 11 x 19 x 6 in. cage) might account for their low level of marking behavior, since the testes growth of *P. m. bairdi* housed under high densities has been shown to be less than that of *P. m. bairdi* housed under low densities (Gardner & Terman, 1970). The average midventral gland size of male *P. m. bairdi* from crowded populations has been found to be smaller than the average gland size of male *P. m. bairdi* from less dense populations (Doty, unpublished data), adding support to this notion. In recent tests with gerbils, males, but not females, were found to be more hesitant in their activity and to mark less frequently in a test arena containing pegs previously marked by another gerbil than in a clean arena (Thiessen, Blum, and Lindzey, 1970). These results suggest that sebaceous sebum inhibits male behavior, while having no noticeable influence upon the female behavior (the possibility of its attractiveness to female gerbils has not been carefully examined).

Marking behavior in *Peromyscus maniculatus gambelii* and *Peromyscus californicus* has been precursorily examined

(Eisenberg, 1962). *P. m. gambelii* have been observed using urine to mark their living areas, depositing it not only in specific corners, but also in other areas of potential contact with strangers, such as in the entrance ways between interconnected cages. Eisenberg (1962, p. 183) states:

> It seems probable that *californicus* uses secretions of the preputial glands in marking objects in the home range, whereas the marking significance of the urine has become secondary. Male *californicus* have repeatedly been observed dragging the perineal region along the floor of the cage after fighting and during the investigation of new areas. This dragging response was not confined to the cage floor, but also occurred on twigs and branches placed in the cage. These same patterns have been observed for *maniculatus*. Whether urine or some other secretion from the anal glands is involved remains a question for further study.

Nicholson (1941) found that wild *P. leucopus* urinate and defecate inside nest boxes he set up in two woodlots near Pinckney, Michigan. Although the mice seemed to leave the cotton nest to micturate primarily in the corners of the boxes, he found that "the moisture remains inside the nest box and is soon absorbed by the cotton nesting material. The urine and fecal pellets together make some of the nests very foul and damp, therefore, in the winter portions of the nest are often frozen solid [p. 204]."

Eisenberg (1963) reports that the behavior of a number of kinds of mice within the Heteromyid family is markedly influenced by areas where perineal dragging, sandbathing, and urination of one or more individuals has occurred. Urination spots in a burrow were found to be thoroughly investigated by intruders. In his test situation, Eisenberg found that the mice tended to investigate and sandbathe at the same spot where one had sandbathed previously. Females tended to bathe less than males, although the functional significance of this is not clear. It would be of interest to examine the female's sandbathing behaviors at various stages of the estrous cycle. In relation to the sandbathing, Eisenberg (1963, p. 41) states, "Sandbathing areas in the wild must be considered as possible areas of information exchange; several animals living in proximity could communicate their individuality and reproductive condition without ever coming into physical contact."

Tieffer (1970) found that both male and female hamsters (presumably Mesocricetus auratus) marked more often when tested with stimulus females than when tested with stimulus males. Hamsters rub flank glands against the walls of a cage in many test situations, and this is generally termed marking.

Basic hormonal and neurological differences between male and female rodents may be present which lend credulance to the proposed role of the female in behaviors related to pair formation and mating. For example, male rats (and some other rodents) are more susceptible to inhibition of sexual behavior by a variety of environmental stimuli than females. When a male is placed with a receptive female in an unfamiliar or previously noxious environment, he will investigate the environment for a prolonged period before paying attention to the female. Completely estrous females, on the other hand, respond promptly to the male's advances and mate without hesitation when placed in a male's home cage (Beach, 1947). In territorial housemice, it has been demonstrated that males, but not females or juveniles, are restrained by the established territorial boundaries (Mackintosh, 1970). Hill (1970) found a very strong tendency (9 of 10 cases) for female _P_. _m_. _bairdi_ to move into an adjacent area inhabitated by a male conspecific. While olfaction has not been carefully examined in producing or helping to produce these effects, it may play a major role. However, Mackintosh (1970) states (p. 182):

> . . . olfaction is unlikely to be important since the heavy traffic in both directions across the boundaries as females and young moved around the enclosure scattered the sawdust thoroughly. Similarly, tactile recognition of local contours would be impossible, hence the orientating clues are likely to be visual.

The present author disagrees with this conclusion, since (as Mackintosh reinterates again and again) territorial males actively patrolled the borders.

In summary, this section has reviewed olfactory studies related to behaviors which potentially influence mate selection, sexual isolation, and dispersion in a number of rodents. The hypothesis has been suggested that, at least in some species, pair-formation and territorial behavior is similar to that seen in a number of pair-forming birds. Divergence of characters related to sexual isolation and pair formation would be expected to be along olfactory, rather than visual, continua. Furthermore, the role of the female rodent in courtship behavior has been stressed. To reiterate the importance of odor in rodent courtship, I have listed below references of articles related to olfaction in rodents which fulfill the criteria for the functions of courtship behavior in birds and fishes, as listed by Bastock (1967, p. 79-85).

Function 1: Synchronization of mating patterns and states. (Whitten, 1956a, Whitten, Bronson, & Greenstein, 1968).

Function 2: Insurance of a safe area for mating. While this function is a byproduct of courtship, which is influenced by olfactory cues, the author knows of no rodent studies which have carefully determined the areas of a home range (either laboratory or wild) where copulation preferably takes place. There is evidence, however, that female mice prefer to give birth in familiar covered nesting boxes, and will withhold labor until alternated to this condition from two hour alternations between such a familiar cage and an open glass fish bowl with strange odor (Newton, Foshee, & Newton, 1966).

Function 3: Bringing the sexes together at the proper time for insemination to occur. (Whitten, 1956a; Le Magnen, 1952; Carr, Loeb, & Dissinger, 1965; Bronson & Caroom, 1971).

Function 4: Prevention of cross-mating between different species. (Godfrey, 1958; Moore, 1965; Smith, 1965).

Function 5: Detering other males from the site of mating. (Eisenberg, 1962; Thiessen, Blum, & Lindzey, 1970).

From this hypothetical beginning, it appears to the present author that many of the untangled and unrelated behaviors noted in olfactory studies are possibly, in some species of rodents, parts of a complex puzzle of behavioral courtship quite similar to that seen in a number of birds and fishes. The following studies were designed with the general purpose in mind to test certain aspects of this theory and to determine in what cases it may or may not be valid.

STUDY 1: PILOT INVESTIGATION

There are a number of limitations to many olfactory studies in relation to ecological and behavioral factors regulating wild populations and influencing speciation. First, the general use of inbred strains greatly limits the validity of the findings with regard to wild populations. This is markedly apparent in light of evidence suggesting that the olfactory identities of housemice (*Mus musculus*) may be greatly reduced or eliminated by inbreeding (Bruce, 1968). Second, in the few studies examining olfactory sexual isolating mechanisms, the relative roles of aversion to heterospecific odors and attraction to homospecific ones have not been examined. Third, in the general context of the paucity of research on interspecific female-mouse behavior, no studies exist which determine if the stage of estrus influences the females' behaviors toward homospecific or heterospecific males or their odors. Theoretically, an olfactory isolating mechanism would only be necessary during times of receptivity, i.e., during estrus.

Within the genus *Peromyscus*, few behavioral studies exist examining isolating mechanisms between the field deermouse, *P. maniculatus bairdi*, and the forest white-footed mouse, *P. leucopus noveboracensis*. While these species are geographically sympatric, they show a tendency to prefer different habitats within their general geographical range. For example, *P. m. bairdi* prefer open habitats (Harris, 1952) and are rarely trapped in woodland or brushland areas (Blair, 1940). *P. l. noveboracensis*, on the other hand, prefer hardwood forests, generally nesting in sites located above ground near the edges of the habitat. In rare instances, members of this species have been found nesting on the ground during the winter, usually when deep snow is present (Nicholson, 1941). Since these species come into contact along adjoining habitats, and have been reported in a few instances, to have nested together during the winter, factors other than ecological ones conceivably contribute to the maintenance of their sexual isolation. According to Hinde's (1959) generalization that divergence is most marked in areas such as the overlap zone of partially-sympatric species, one might expect divergence in behavioral and/or morphological characters related to olfaction between these two species. Morphological differences in relation to a midventral sebaceous area have been recently documented for these species (Doty and Kart, 1972).

Bradshaw (1970) presented some preliminary evidence suggesting that female forest deermice (*Peromyscus maniculatus nubiterrae*, preferred to nest near caged-off male conspecifics than near caged-off male *P. leucopus*, although the results were not statistically significant. Bradshaw also found that female *P. leucopus* preferred to nest near male *P. maniculatus nubiterrae*, contrary to logical expectations. Bradshaw did not examine the females' stages of estrus. As mentioned in the introduction, Hill (1970) found evidence that *P. m. bairdi* females tend to prefer to move into nests with homospecific males (thereby exhibiting a female selection of a mating partner), whereas *P.l. noveboracensis* females show no such tendency, with half or so not even nesting with nearby males. A strong tendency for *Peromyscus leucopus* to nest individually during breeding season has been reported by Nicholson (1941). Nicholson found *P. m. bairdi*, on the other hand, generally nested in bisexual pairs.

The primary purposes of Study 1 were as follows:

(a) to determine if differential preferences exist in female *P. maniculatus bairdi* mice for odors of male *P. maniculatus bairdi* and *P. leucopus noveboracensis* mice as a function of estrus and diestrus produced by the administration of exogenous hormones;

(b) to examine such preferences periodically throughout a full 16-hour testing period extending from 7:00 p.m. to 11:00 a.m. the following day, in order to potentially ascertain whether circadian factors are present which may influence individual preferences in unique or systematic ways, and whether these factors, if present, are independent of circadian running patterns; and

(c) to work out procedural, methodological, and apparatus problems.

Method

Subjects

Three six- to eight-month old female *P. maniculatus bairdi* mice served as *Ss*. All Ss were descendants of mice trapped in Central Michigan and had been in the colony for less than six generations.[5] The *Ss* were weaned at 21 days of age, housed in small like-sexed groups in plastic laboratory cages (28 x 17 x 13 cm.), provided with wood-chip bedding and given Purina lab chow and water *ad libitum*. The stimulus animals, i.e., the animals used to produce the experimental odors, included four adult male mice: two *P. maniculatus bairdi* and two *P. leucopus noveboracensis*. All female *Ss* were ovariectomized in adulthood at least 10 days before testing. When tested under the "estrus" condition, they were rendered sexually receptive by single daily subcutaneous 10 to 20 μg. injections of 17 Beta Estradiol in 0.1 cc. sesame oil for three days before testing, followed by a single 500 μg. injection of progesterone

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[5] The *P. maniculatus* and *P. leucopus* were kindly provided by John King, Department of Zoology.

approximately 6 hours before the test.[6] "Diestrus" testing sessions were preceded by the same injection sequence of sesame oil only.

Apparatus

The acute problems of odor control and response measurement led to the creation of the rodent olfactorium pictured in Figure 1. The testing portion of the olfactorium consisted of an aerodynamically-shaped plexiglas box, designed to insure proper airflow and maximally effective

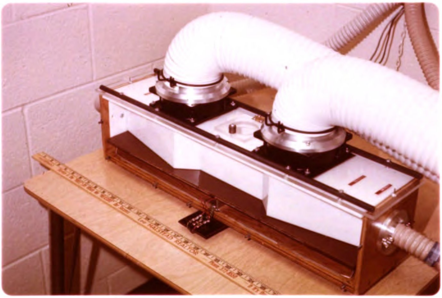

Figure 1. Rodent olfactorium

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[6] I would like to thank Linda Coniglio, Sue Porges, and Lyn Clemens for their considerate help and advice on these procedures, as well as for the provision of their laboratory facilities. Thanks are also extended to Gordon Duncan of The Upjohn Company for providing some of the hormones.

flow rates of odor stimuli. The internal height of the chamber was 90 mm. The internal lateral dimensions of the test chamber are presented in millimeters in Figure 2. In this Figure, the 55 mm. diameter circles represent the holes through which incoming air was exhausted, and the 26 mm. radius circle represents the hole through which the animal was introduced at the beginning of a test session. This hole was covered by a plexiglas door during a test session. Stimulus odors could be introduced into the far ends of the apparatus through custom-lathed aluminum sleeves attached to 1 1/4 in. I.D. nylon hoses connected to plexiglas stimulus-animal cages located in an adjacent room (Figure 3). Air from the adjacent room's fresh air ventilation system could be forced through the stimulus animal cages and into the ends of the apparatus. A stimulus animal was allowed to live in each of the stimulus-animal cages for a number of days before testing was begun, to insure an accumulation of urine and feces. The stimulus animals were present in these cages (one per cage) during testing. Two whisper fans located in the roof of the apparatus exhausted the incoming air through 55 mm. I.D. ducts into the buildings direct-exit ventilation duct, which was independent of the fresh-air system. The design of the air-flow system confined the flow of each odor source primarily to the half of the apparatus into which it was introduced. The floor of the olfactorium was constructed of electronic panelboard,

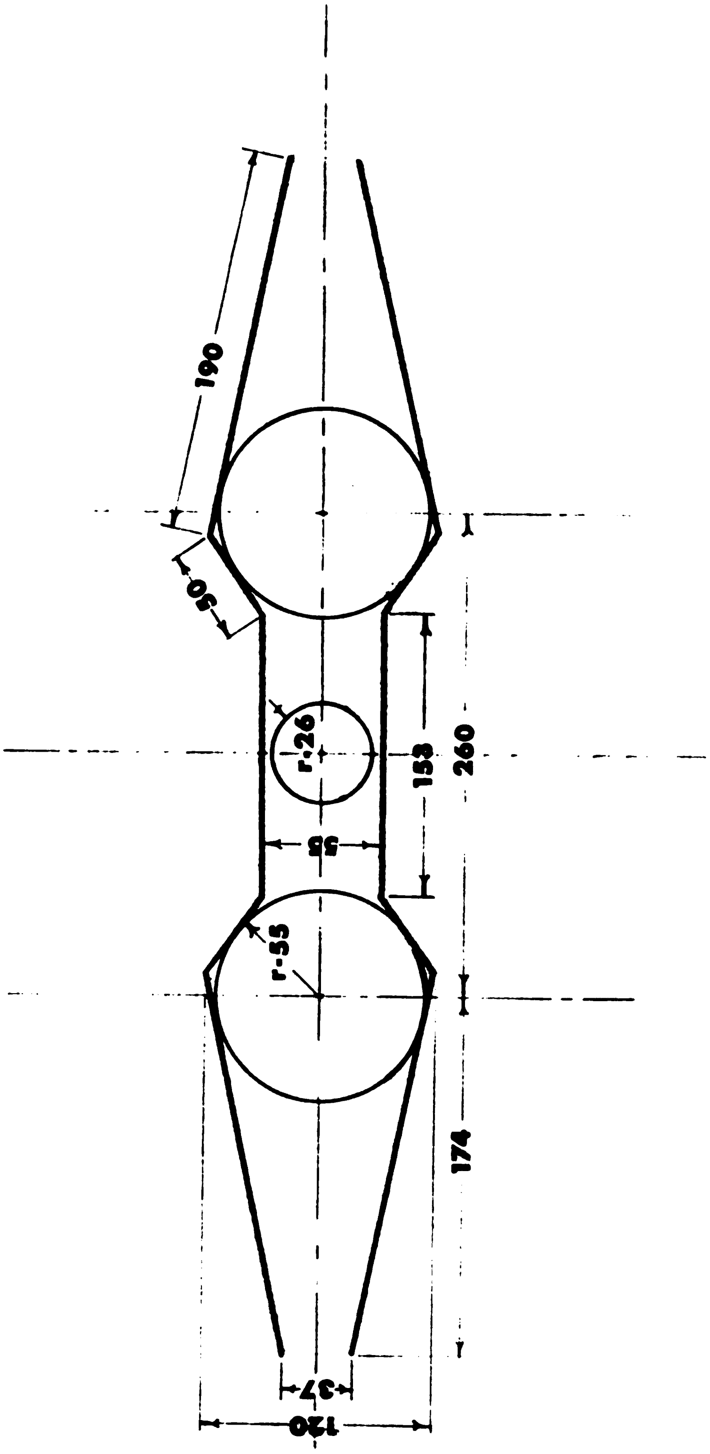


Figure 2. Internal lateral dimensions (in millimeters) of test chamber.

which was relatively impervious to moisture and resultant warpage, and could be easily cleaned of odorous substances. The entire floor pivoted on a centrally-located aluminum fulcrum, allowing approximately 0.5 cm. total vertical movement at its ends. A 2 in. high plastic hurdle separated the compartment into two distinct portions. The S's weight at either side of the pivot point and hurdle closed a semi-silent switch which allowed recording of the mouse's barrier crosses and the time spent in either compartment on both event recorders and cumulative clocks located in the stimulus-animal room (Figure 3). Other pieces of electronic equipment (e.g., relays), as well as the stimulus animal cages, were also located in this room, to eliminate as many

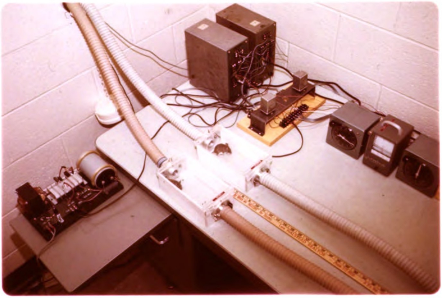


Figure 3. Stimulus animal cages
and recording equipment.

extraneous noises as possible from the test situation. Separate nylon intake hoses were used for each stimulus odor, so that cleaning of the hoses was not necessary during the experiment. At each end of the apparatus, a single Purina mouse pellet was clipped to the ceiling by means of an allegator-type clamp, and water was provided via watering tubes extending from graduated cylinders into each end of the olfactorium.

Procedure

At the time of testing, each S was brought in its home cage from the colony room and transferred to the center placement hole of the olfactorium. S 2 was placed into the apparatus at 6:30 p.m. each day, whereas the other two Ss were placed into the test situation only a few minutes before 7:00 p.m. Since the test room was maintained on a 12:12 light-dark cycle, with lights off at 7:00 p.m., S 2 received a longer daily initial experience in the test situation with the lights on than did the other Ss.

Each mouse was tested for four consecutive nights under either the "estrous" or "diestrous" condition, followed by four consecutive nights under the other condition. Ss 2 and 3 received the diestrous condition first, whereas S 1 received the estrous one first. To insure the presence of high levels of ovarian hormones during the estrous test days, a daily 10 to 20 μg. injection of estradiol benzoate (in 0.1 cc. sesame oil) followed 3 to 6 hours later by an injection of 500 µg. progesterone (in 0.1 cc. sesame oil) was given to each S, even though previous injections to produce estrus had been given (page 40). Since S 1 received the estrous condition first, six days were allowed to elapse between the last hormone injection and the first day of diestrous testing, to allow a decline in the estrogen and progesterone hormone levels. Testing was continued until 12:00 noon each day, when the S was returned to his home cage and the colony room. The directions of the odor presentations were systematically counterbalanced [left (L) or right (R) homospecific odor] for each S as follows: S1, RRLLRRLL; S2, LRLRLLRR; and S3, LRRLLRRL.

Results

The data are summarized for each S in Figures 4, 5, and 6. Each data point is the mean of four one-hour periods under a common hormonal state, i.e., "estrus" or "diestrus." Thus, each figure is based upon 128 hours of testing. It is apparent that the Ss spent more relative time on the homospecific male-odor side when in estrus, and surprisingly, more relative time on the heterospecific side when in the diestrous condition. Due to the nature of a forced-choice test situation, it cannot be concluded that the Ss preferred the heterospecific odor while in diestrus,

Figure 4. Percent time with homospecific odor for *S* 1.

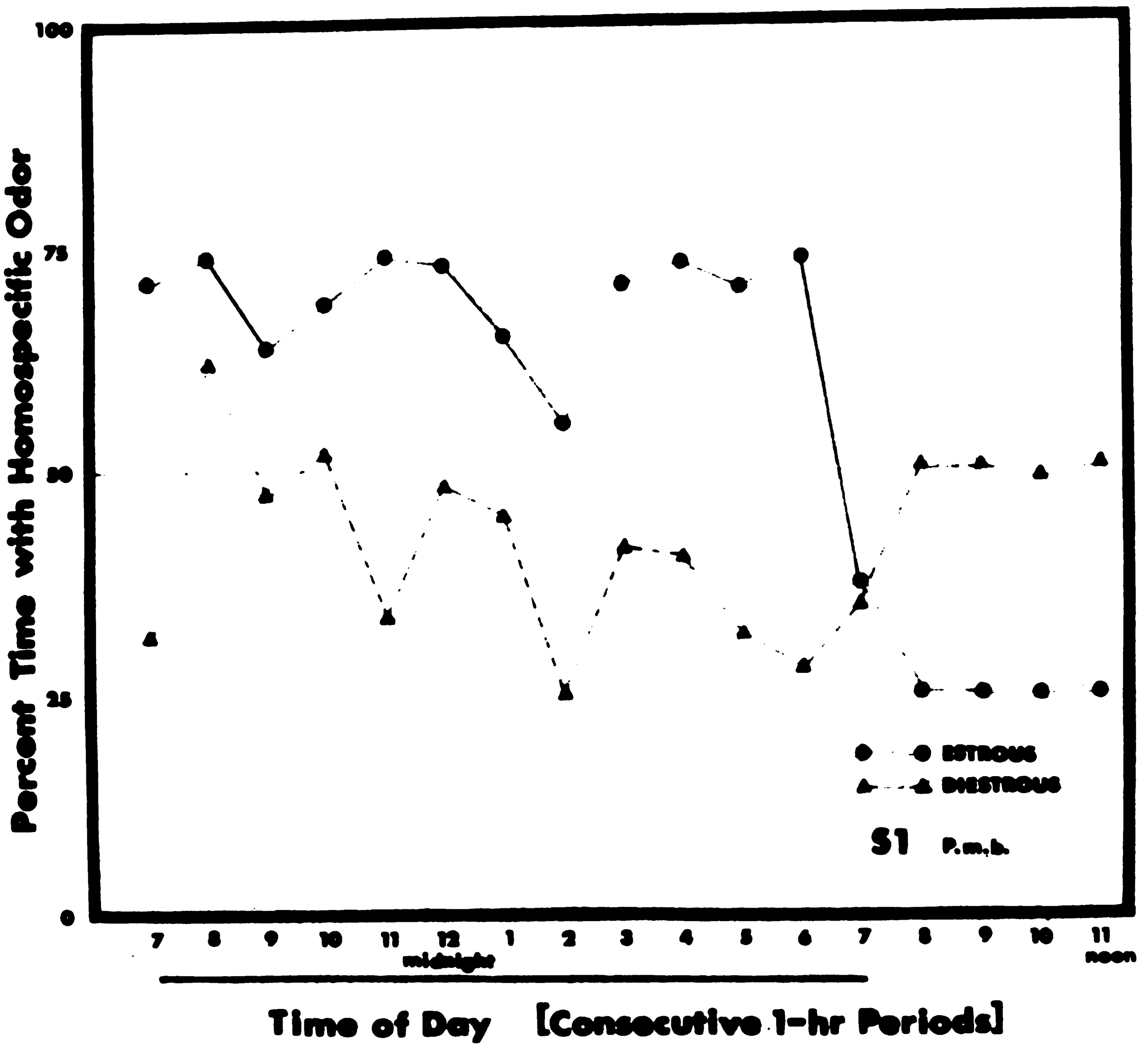

Percent Time with Homospecific Odor
0
25
50
75
100
7
8
9
10
11
12
midnight
1
2
3
4
5
6
7
8
9
10
11
noon
Time of Day [Consecutive 1-hr Periods]
● —● ESTROUS
▲- - -▲ DIESTROUS
S1 P.m.b.

Figure 5. Percent time with homospecific odor for S 2.

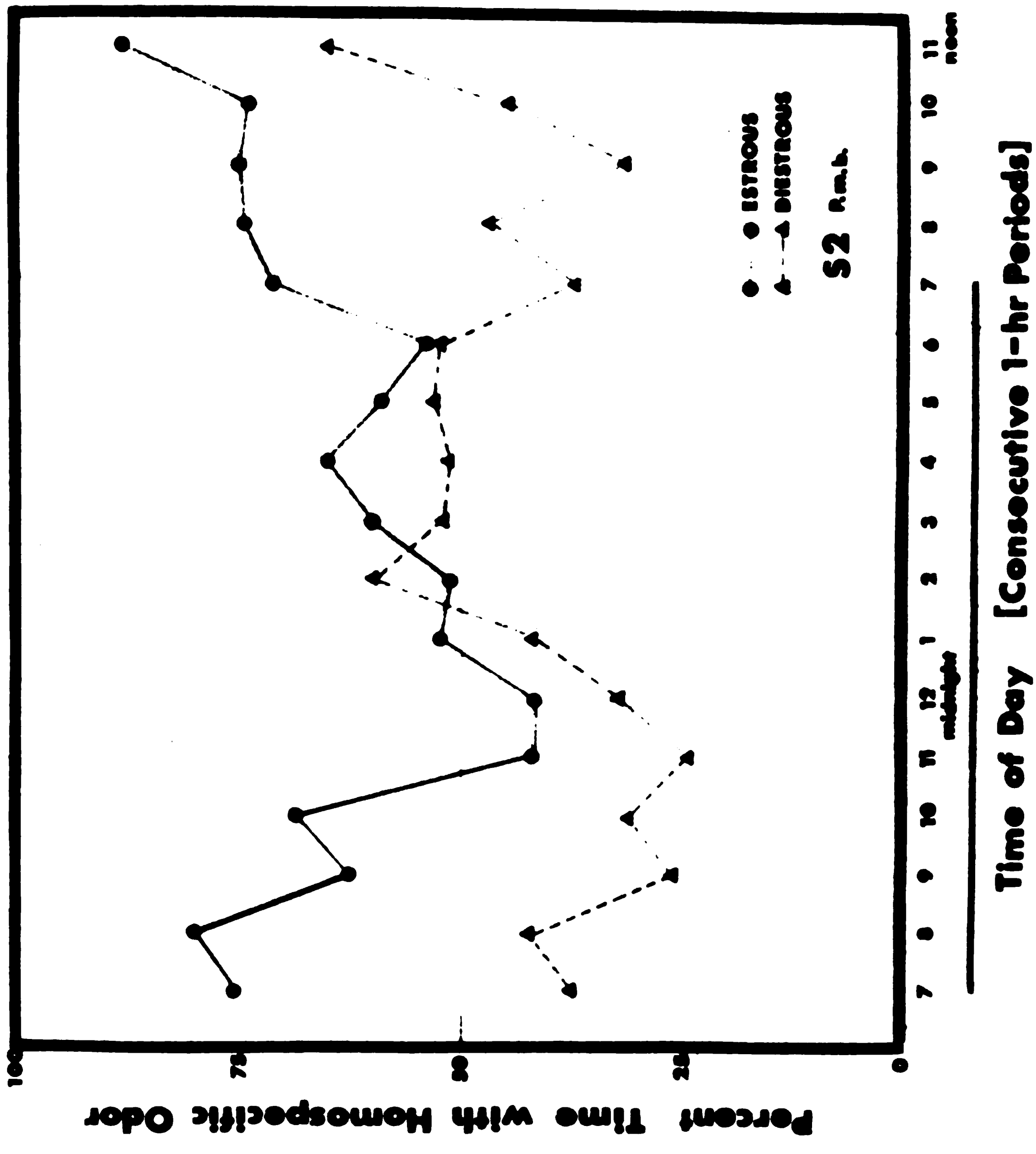
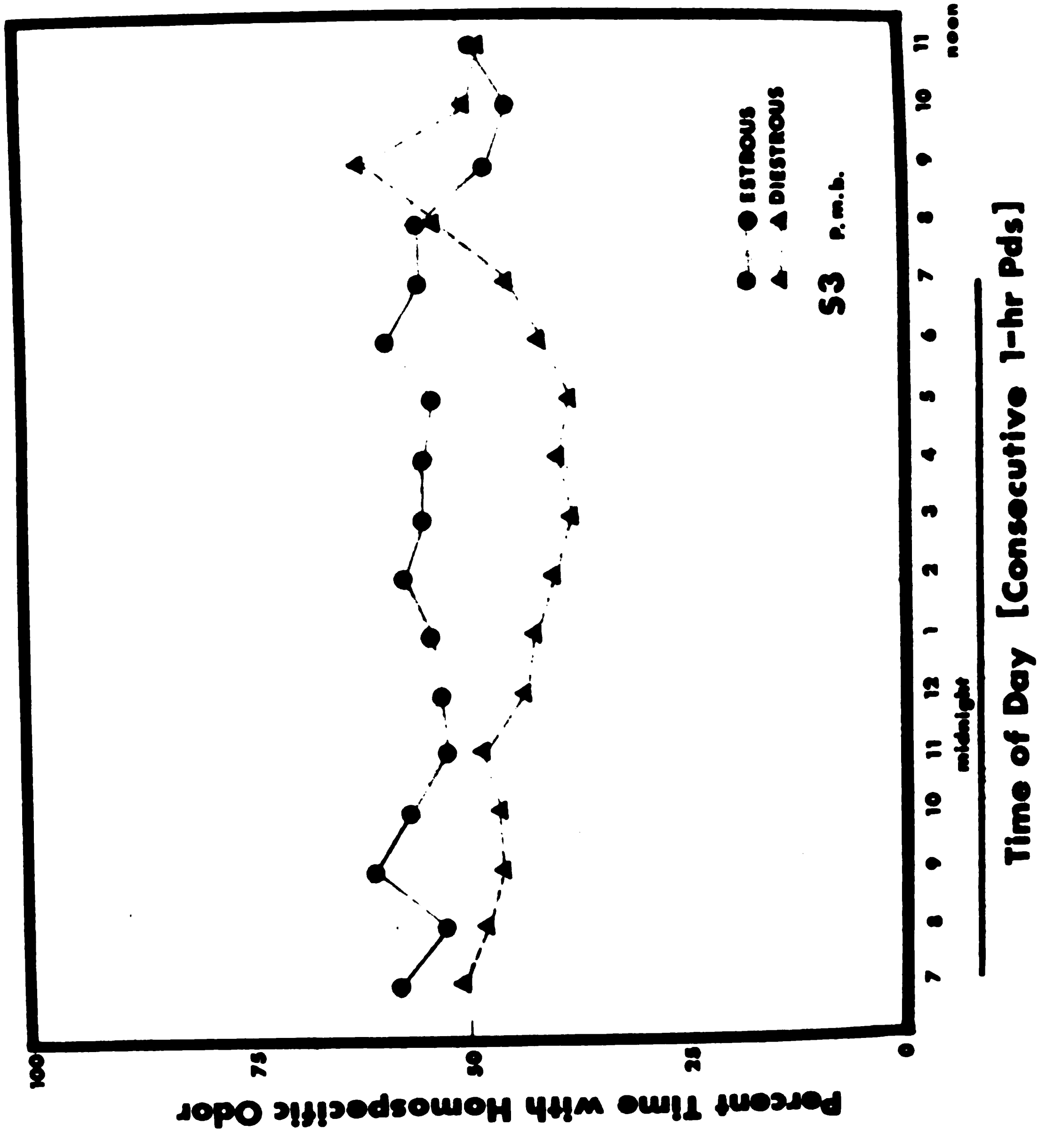

Percent Time with Homospecific Odor
0
25
50
75
100
7
8
9
10
11
12
midnight
1
2
3
4
5
6
7
8
9
10
11
noon
Time of Day [Consecutive 1-hr Periods]
● — ● ESTROUS
▲ — ▲ DIESTROUS
S2 R.m.b.

Figure 6. Percent time with homospecific odor for *S* 3


Percent Time with Homospecific Odor
0
25
50
75
100
7
8
9
10
11
12
1
2
3
4
5
6
7
8
9
10
11
midnight
noon
Time of Day [Consecutive 1-hr Pds]
● ESTROUS
▲ DIESTROUS
53 p.m.b.

however. No statistical analyses were performed on these data due to the small number of Ss and the exploratory nature of the study.

Extremely large individual differences in hurdle crossing by the three Ss undoubtedly influenced the behavior graphed in Figures 4-6. S 3 was so adept at hopping back and forth at the center of the apparatus that thousands of crosses were made in most of the half-hour intervals, and a reliable estimate of her barrier crosses during most of the periods could not be made. In contrast, S 1 rarely made more than 6 or 8 crosses in any given half-hour interval. A comparison of Figure 4 with Figure 6 shows the net effect of such high activity--a diminuation of obtained behavioral differences between the estrous and diestrous conditions in the testing situation. Figure 5 shows this effect within a single S. Under high periods of barrier crossing, it is extremely likely that only short periods of rest influenced the data, contributing little to the preference determination.

No easily-demonstrable learning effects were present in the data. The small number of Ss, different odor-combination counterbalancing across Ss, heterogeneous activity levels, and varying estrus-diestrus testing orders make determination of such factors, if present, nearly impossible.

The Ss consistently preferred the right of the olfactorium to the left, independent of odor preferences. This may or may not have been due to the side of the initial encounter with the odor.

Discussion

The male odor-preference differences of the female *P. maniculatus* as a function of estrous state are noteworthy. The behavior of the diestrous females is indicative of either (a) a preference for heterospecific odors over homospecific ones; (b) an aversion to or avoidance of homospecific male odors; or (c) some combination of (a) and (b). If avoidance of the homospecific odor, or attraction to the heterospecific one, consistently occurs in diestrus mice, then the findings of Bradshaw (1970) which suggested female *P. leucopus* preferred to nest near heterospecific males (*P. m. nubiterrae*) may be due to the fact that female mice are usually in diestrus 3/4 or 4/5 of the time (at minimum). Eisenberg (1963) reported that diestrous mice of a number of species within the Heteromyid family exhibited marked escape and avoidance tendencies when tested with male conspecifics, but these tendencies disappeared when they were in estrus. Perhaps smell is one sensory avenue mediating these different behaviors toward males, as these data tend to suggest.

Another important finding of this study is that female *P. maniculatus* preferred the homospecific-odor side to the heterospecific one only during estrus. The present data, along with those presented by Carr, Loeb, and Dissenger, (1965), suggest the importance of the estrous cycle in influencing odor preferences in some rodent species.

There are a number of limitations to Study 1. First, the small number of Ss is probably not representative of a larger population. Second, long test periods theoretically may produce marked adaptation and learning effects. Due to the small number of Ss, high circadian activity during some periods, and different counterbalancing orders, however, it is difficult to determine if these factors were important. It might be possible, for example, that the waxing and waning of the curves in Figures 4 and 5 are the result of adaptation. It is of interest that the estrous and diestrous curves of S 1 tend to parallel each other during the 8:00 to 10:00 p.m. and the 12:00 to 4:00 a.m. periods. The estrous and diestrous curves of S 2 also tend to parallel each other during the 7:00 to 10:00 p.m. period. A close examination of the data suggested, however, that no clear uniformity from day to day existed in regards to these factors, and that marked position preferences formed on day 1 may have influenced the Ss' subsequent performance. This is in accord with Moore's (1965) observations that his Ss tended to return to the portion of the test situation in which they had spent their first recorded period of inactivity. Third, the use of ovariectomized rather than naturally-cycling females may result in unknown effects upon systems which may directly or indirectly confound the Ss' preferences. However, this procedure is commonly used in a number of instances where precise control of gonadal secretions is required, and, as the next study suggests, probably has little effect upon the dependent variable. Fourth, the long test periods may have resulted in the confounding of the dependent variable with behaviors independent of, or non-predictably correlated with, the olfactory preference. Examples of these include grooming, sleeping, running, eating, etc. It is probable, however, that the repeated measurements design and the counterbalancing of the side of the odor presentations randomized the majority of these effects across both odor conditions. Fifth, early position effects may have been learned which lessened the magnitude of the observed overall preference measures. An examination of Day 1's data for all three Ss suggested this may have occurred, although this cannot be determined due to the small number of Ss. Furthermore, the position preferences may not be dependent upon the previous days' tests. The strong position preference of all Ss for the right side of the apparatus might also be due to the possibility that the floor on the left side was slightly "springier" than that on the right. Sixth, the present design did not completely eliminate possible auditory cues from the stimulus animals which may interact with olfactory ones in the test situation.

The testing of a rather small number of Ss over a comparatively large number of sessions was predicated on the belief, in this instance, that the aforementioned difficulties were minimal, and that the advantages of within subject comparisons over longer test periods outweighed the disadvantages. The present design resulted in large amounts of data on the same subject gathered at different test sessions, thereby yielding comparatively reliable effects not directly dependent upon a single test session. The within-subject design allowed partial control of ideosyncratic factors, and the longer testing minimized influences such as the Ss' initial reactions to their being handled with tongs. However, as the next study indicates, shorter test periods may be preferable to longer ones.

The following conclusions can be made from Study 1 and will be incorporated in the next, more definitive, experiment:

(a) Since the preference measure was confounded by periods of circadian running activity, the most preferable test times appear to occur between 7:00 p.m. and 10:00 p.m.;

(b) The possible development of position preferences in the test apparatus suggest that an independent groups design may be preferable to a repeated-measurements one;

(c) Testing should be carried out in darkness, with no subjects receiving experience in the test situation during a lighted period;

(d) data from naturally-cycling females should be collected in insure external validity (Campbell & Stanley, 1966) of the findings. Consideration of these conclusions from Study 1 led to the design of Study 2.

STUDY 2: ODOR PREFERENCES OF FEMALE *P. MANICULATUS* FOR MALE-MOUSE ODORS OF *P. MANICULATUS* AND *P. LEUCOPUS* AS A FUNCTION OF ESTROUS STATE[7]

The purpose of Study 2 was to examine olfactory preferences of naturally-cycling *P. m. bairdi* females for male-mouse odors of *P.l. noveboracensis* and *P. m. bairdi* as a function of estrous state in an independent-groups design utilizing short test sessions.

Method

Subjects

Twenty sexually-naive female *P. m. bairdi* (mean age = 93.11 days) served as *Ss*. All *Ss* were laboratory born and raised, and were weaned at 21 days of age. Following their transportation from the Biology Research Center to our laboratory, they were housed individually in plastic (28 x 17 x 13 cm.) mouse cages provided with wood-chip bedding and cotton nesting material. Food and water

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[7] The results of Study 2 were presented at the Animal Behavior Society at their winter meeting with the American Association for the Advancement of Science in Chicago, Dec., 1970, and are published in abstract form (Doty & Levine, 1970).

were available ad libitum, and the Ss were maintained on a 12:12 light-dark cycle with lights off at 7:00 p.m.

Apparatus

The experimental apparatus was essentially the same as that described in Study 1, with the following modifications: (a) A counterbalanced rocker-door was installed between the two halves of the olfactorium in place of the previous full-tilt floor (see Dice, 1961, p. 164, for a description of a similar two-position treadle with weighted arm and two barriers); (b) a 2-in. I.D. 12-in. long tube was attached to the center placement hole of the olfactorium to eliminate any possible initial placement bias of the mouse by E.

Procedure

The Ss were randomly divided into two experimental groups, termed "estrus" and "diestrus." Vaginal smears were taken twice daily--once in the morning and once before the test--and all Ss were categorized, as closely as possible, into one of five stages of estrus listed by Allen (1922): (a) diestrus, where the smear consists primarily of leucocytes and stringy vaginal material; (b) proestrus, where nucleated epithelial cells dominate the smear; (c) estrus, where cornified (squamous) epithelial cells without nuclei predominate; (d) metaestrus I, where the cornified elements are bunched or caked; and (e) metaestrus II, where an increase of leucocytes gradually overshadows the horney elements. Due to the cycle's continuous nature, and problems inherent with repeated vaginal smearing in these highly emotional animals, estrus was the easiest smear type to categorize. Some difficulty was encountered, in a number of instances, in distinguishing between the other smear types. An attempt was made, however, to examine previous days' cyclings in order to best categorize the smear. Whenever possible, a S was tested only when both cycling expectation and smear type were in agreement. Ss with regularly occurring cycles (approximately 5-day) were the easiest to classify, while some females rarely became estrus, remaining diestrus (pseudopregnant) for long periods of time. In a small number of instances, a non-cycling female of the "estrus" group was exchanged with a cycling one of the "diestrus" group. In no instance was a S exchanged which had previously been tested in the olfactorium.

The sides of the odor presentations were systematically counterbalanced for both groups. Test sessions were run between the hours of six o'clock and midnight, and lasted exactly one hour and 15 minutes. In the independent groups design, each S was tested only once in the olfactorium. Between each test session, the apparatus was taken apart and thoroughly washed with a hot, soapy mixture of water and a detergent-germicide deodorizer, "Mikro-Quat," whose major ingredients include alkyl, dimenthyl benzyl ammonium chlorides, and trisodium ethylenediaminetetraacetate.

The total time spent on each side of the apparatus, as well as the number of crosses, was determined for 15 minute segments of the 1 1/4 hr. test by an examination of the Rustrak printouts. The division of the test period into five equal segments was made to allow intratest reliability analysis, as well as to examine possible learning or adaptation effects. All tests were made in darkness, with the S being inserted down the tube at the center of the olfactorium. The rocker-door was balanced before this insertion, to eliminate possible placement bias. The Ss were always grasped with tongs by the tail and transferred immediately from their nesting cage located in a cart with a plywood holding box attached on top into the experimental apparatus. Following testing, each S was returned to its home cage and wheeled back into the colony room.

Results

The results of the 1 1/4 hour sessions are presented in Table 1. The diestrous Ss showed a slight preference for the heterospecific odor side, although this did not differ significantly from the expected 50:50

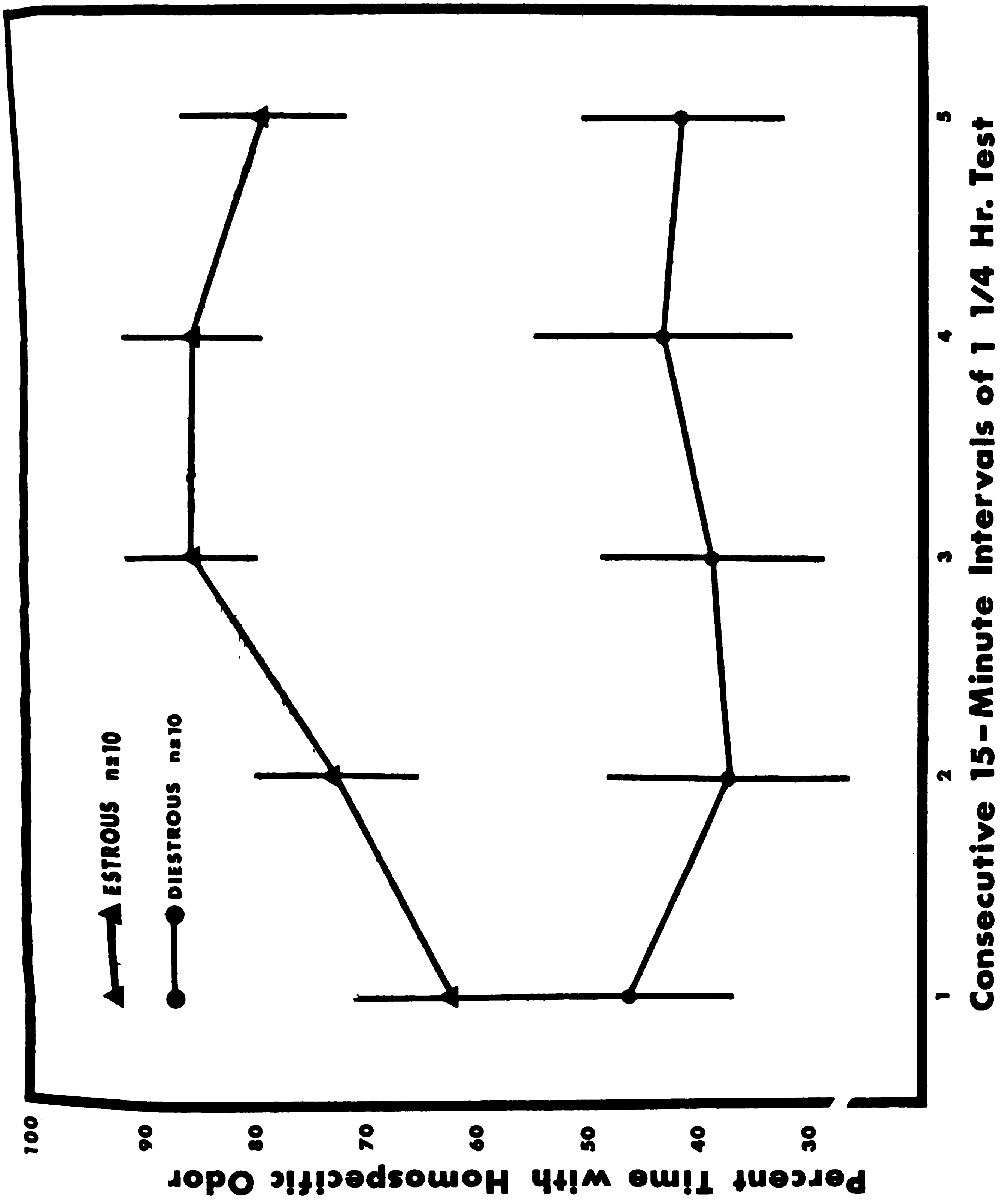
distribution (Binomial Test, $p > .10$). However, the estrous Ss showed a strong preference for the homospecific-odor side (Binomial Test, $p < .001$). The variances of the percentage values did not depart significantly from homogeneity ($F_{max} = 2.66$, $p > .10$). A t-test between the means of these percentage values for the estrous and diestrous groups (77.6% vs. 41.5%) proved highly significant ($t = 7.04$, $df = 18$, $p < .0005$), as did a U-test between the medians (74.5% vs. 38.0%; Mann-Whitney $U = 13$, $p < .01$). Neither the mean nor the median number of crosses, however, differed significantly between the two groups ($t = 0.51$, $df = 18$, $p > .10$; $U = 47$, $p > .10$).

Table 1. Percent time spent with homospecific odor and number of crosses in olfactorium during 1 1/4 hr. test for estrous and diestrous *P. maniculatus* (heterospecific odor = *P. leucopus*).

	% Time with Homospecific Odor			# Crosses		
Group	Median	Mean	S.D.	Median	Mean	S.D.
Estrus (n=10)	74.5	77.6	16.1	54.0	143.2	236.2
Diestrus (n=10)	38.0	41.5	26.2	52.0	172.3	245.8

The mean percentages of time spent with the homospecific odor for both estrous and diestrous groups are presented in Figure 7 as a function of consecutive 15-minute intratest intervals. Each vertical line is plus and minus

Figure 7. Percent time spent with homospecific odor.


ESTROUS n=10
DIESTROUS n=10
Percent Time with Homospecific Odor
100
90
80
70
60
50
40
30
1
2
3
4
5
Consecutive 15-Minute Intervals of 1 1/4 Hr. Test

one standard error of the mean. It should be noted that the reduced variance seen in the estrous group is due, in part, to the restricted range of extreme percentage scores. However, as mentioned earlier, the variance of the diestrous group did not exceed that of the estrous to the extent of heterogeneity.

The medians for each 15-minute intratest segment are listed for both the estrous and diestrous groups in Table 2. The values of Table 2 are in close agreement with the means of Figure 7.

Table 2. Median percent time spent with homospecific odor for estrous and diestrous _P. maniculatus_ during 15-minute consecutive intervals of 1 1/4 hr. test.

	Consecutive 15-minute Intervals of 1 1/4 hr. Test				
Group	1	2	3	4	5
Estrous (n=10)	64.5	66.5	93.0	97.0	83.0
Diestrous (n=10)	53.0	40.0	39.0	49.0	45.0

The mean and median number of crosses for both the estrous and diestrous groups in each of the consecutive 15-minute test segments are presented in Table 3.

The large discrepancies between the mean and median values of Table 3 suggest that high activity of a few _Ss_ in both the estrous and diestrous groups inflated the size of the means, and that the medians are more representative of the sample's crosses. This is also true for the number of crosses during the total 1 1/4 hr. test session (Table 1).

Table 3. Mean and median number of crosses in olfactorium for estrous and diestrous P. maniculatus during consecutive 15-minute intervals of 1 1/4 hr. test.

	Consecutive 15-minute Intervals of 1 1/4 hr. test				
Group	1	2	3	4	5
Estrous					
Mean:	41.8	33.3	13.4	22.3	41.6
Median:	28.0	13.0	4.0	2.5	0.5
Diestrous					
Mean:	41.0	14.8	14.1	48.8	53.9
Median:	27.5	10.0	8.5	8.0	5.5

It is apparent from Table 3 that both the estrous and diestrous groups show a marked decrease in the median number of crosses across segments of the test period. It is unlikely that this decrease effected the preference scores differentially for the estrous and diestrous groups, as neither the mean nor the median number of crosses were significantly different between them for the entire test sessions, as indicated above. Furthermore, the estrous group crossed slightly more often than the diestrous one during the first two segments of the test, while the reverse was true during the last three segments. This reversal had no apparent effect upon the marked differences between the preferences of both groups (Figure 7), which are present across all five test segments.

By the nature of a two-category preference task, a negative correlation would be expected to occur between the number of crosses and the percent time spent on one side of the apparatus, if one side was consistently preferred over the other. This is apparent between the number of crosses and the percent time spent on the homospecific side in the estrous group (Spearman Rank $r = -.70$, $p < .05$), but not between these variables for the diestrous one (Spearman Rank $r = +.20$, $p > .10$). From the aforementioned considerations, it is most likely that this correlation arises from the nature of a two-category task, rather than from a simple cause and effect relation between activity and odor preferences. In fact, a preference might be conceptually defined, in the present experiment, as a decrease in the number of crosses with a concomitant increase in the amount of time consistently spent on one odor-side of the olfactorium over that of the other.

In order to determine the reliability of the Ss' percent time spent with the homospecific odor within the 1 1/4 hr. test session, rank-order correlation coefficients were determined between the 15-minute test segments plotted in Figure 7. These correlations are presented for the estrous and diestrous groups in Tables 4 and 5, respectively.

The smaller reliability coefficients of the estrous group in relation to the diestrous one is most probably due to a larger number of tied ranks (15 of 50 ranks in the estrous group were tied, whereas only 9 of 50 were tied in the diestrous one).

Table 4. Rank-order intratest reliability coefficients for percent time with homospecific odor in estrous _P_. _maniculatus_.

Segment	1	2	3	4	5
1	--	.89**	.52	.39	.26
2		--	.56*	.64*	.51
3			--	.37	.47
4				--	.88**
5					--

**p< .01
*p< .05

Table 5. Rank-order intratest reliability coefficients for percent time with homospecific odor in diestrous _P_. _maniculatus_.

Segment	1	2	3	4	5
1	--	.92**	.85**	.49	.59*
2		--	.97**	.56*	.60*
3			--	.59*	.63*
4				--	.88**
5					--

**p< .01
*p< .05

Discussion

The present results suggest that female P. maniculatus reactions to male-mouse odors are significantly influenced by gonadal state in a two-odor, forced-choice preference situation. In general, the results confirm those found in Study 1. Furthermore, the present study indicates that marked odor preferences are exhibited by the P. maniculatus in a relatively short test period.

Although the present study implicates the importance of the estrous cycle in female P. maniculatus preferences for the homospecific male-mouse odors, it is not clear whether the homospecific preference is due to attraction to the homospecific, avoidance of the heterospecific, or some combination of both odor situations. It may well be, for example, that an estrous female would be attracted to the odor of a heterospecific male in the absence of the homospecific-male odor. Furthermore, a diestrous female may avoid the heterospecific as well as the homospecific odor. The data of Study 2 might be used to support this latter contention, in that a marked preference for the heterospecific side was not shown by the diestrous Ss. This conjecture, however, is premature until more conclusive data have been obtained.

The use of stimulus odor eminating from intact males and their excreta makes the determination of the exact stimulus mediating the avoidance and attraction difficult. *P. m. bairdi* male mice, for example, possess a sebaceous midventral gland which presumably secretes odoriferous substances, while male *P.l. noveboracensis* possess no such gland (Doty & Kart, 1972). Furthermore, mature *P. leucopus* males have been observed to have a higher water intake and urine excretion rate than mature *P. m. bairdi* males in a metabolism-cage situation (Doty, personal observation), but it is not clear if this difference is due solely to the larger body size of *P. leucopus* males. This finding is not in agreement with other observations of water intake in these species, which usually find that *P. maniculatus* have greater water intake (cc/g/day) than *P. leucopus* (e.g., Lindeborg, 1952; Odum, 1944). However, regardless of the direction of the difference, differential amounts of stimulus urine and feces could potentially influence the attractiveness of one or the other of the odors. While it is not currently possible to equate the species stimuli on any meaningful physical odor dimension, perhaps similar amounts of urine and fecal material would be a conceptually valid start. On the other hand, one might argue that the amount of material given off by a single individual in a given time period might conceptually be a more valid way to equate stimuli factors. The present author favors equating the stimulus material along some physical dimension, such as volume.

STUDY 3: HOMOSPECIFIC AND HETEROSPECIFIC MALE URINE ODOR PREFERENCES IN FEMALE *P. MANICULATUS* AND *P. LEUCOPUS*

Studies 1 and 2 have established that estrous female *P. maniculatus* mice spend more time on the side of a two-choice olfactorium containing the odor of a homospecific male than on one containing the odor of a male *P. leucopus*. Diestrous *P. maniculatus* on the other hand, showed no such preference. While these findings are interesting, they are limited in several ways. First, the response to one odor may be strongly influenced by the presence of the second odor. For example, one test odor may (a) change the physical characteristics of the other odor (possibly through mixing) and/or (b) alter the sensory system(s) of the subject in a manner affecting the odor perception. Second, the observed preference cannot be isolated as an avoidance of one odor complex, attraction to the other, or some combination of both avoidance and attraction. Third, the stimulus complex of an animal and its excreta may be unrepresentative of stimuli encountered temporally or spatially in most wild situations. For example, urine and feces may be associated with territorial or home range boundaries, but not with (i.e., in combination with) odors produced by the animal (e.g., sebaceous or sudoriferous odors). Fourth, the presence of stimulus animals in small collecting cages during testing increases the likelihood of nonolfactory cues (e.g., sounds) in confounding the preference. Furthermore, housing of the stimulus animals in a small cage may facilitate the production of a presumed urinary fear substance similar to that described in *Mus musculus* (Carr, Martorano, & Krames, 1970; Müller-Velten, 1966).

To minimize these problems, Study 3 utilized urine odors and the pairing of the stimulus odor with air rather than with another odor. Urine odor was chosen as the stimulus for a number of reasons, some of which were mentioned in the introduction. First, urine has been implicated in the marking of territorial and home range boundaries in a number of mammals (Hediger, 1968), including *Peromyscus* (Eisenberg, 1962). Second, there is experimental evidence that conspecific estrous female urine is attractive to male rats (e.g., Carr & Pender, 1958; Pfaff & Pfaffman, 1969) and dogs (Beach & Gilmore, 1949). Reiff (1956) reported that laboratory rats of both sexes showed a marked interest in opposite-sex urine, with old "fermented" urine being particularly attractive, and fresh urine being much less so. However, this work has been carefully criticized on a number of points by Schultz & Tapp (1971).

Third, Scott and Pfaff (1970) have demonstrated that female *Mus musculus* are attracted to male conspecific urine. Bronson and Caroom (1971) present evidence that preputial secretions are deposited in the male urine of *Mus musculus*, and have found such urine to be highly attractive to females. Fourth, male urine has been implicated in estrus synchrony of female mice (Bronson, 1971), in producing pregnancy blockage in recently inseminated female mice (Dominic, 1966), and in increasing activity and aggression in male mice (Archer, 1968; Lee, 1970; Mackintosh & Grant, 1966; Ropartz, 1968b). Fifth, female mouse urine (either estrous or diestrous) has been found to decrease aggression in male *Mus musculus* (Mugford & Nowell, 1970, 1971a). While the reliable deposition of urine in relation to handling by a gloved experimenter has been demonstrated in several *Peromyscus* species (Doty, 1971), little work has determined the functional utility of such responses in wild situations.

The purpose of Study 3 was to examine female mouse behaviors toward the odor of cumulatively-deposited male-mouse urine as a function of the species of the female *S* (*P. maniculatus* or *P. leucopus*), the species of the male mice producing the urine (*P. maniculatus* or *P. leucopus*), and the gonadal state of the female *S* (estrus or diestrus).

Method

Subjects

Thirty-two female *P. maniculatus bairdi* and 32 female *P. leucopus noveboracensis* served as subjects. At the time of testing, the ages in days (mean ± S.D.) of these two groups were 74.75 ± 31.79 and 49.81 ± 13.26, respectively. The *Ss* were separated at weaning (21 days) into like-sexed conspecific groups of two to six animals, and housed in 28 x 13 x 13 cm. plastic cages containing wood chip bedding and nesting material. Purina mouse pellets and water were provided *ad libitum*. Following transportation from the Biology Research Center to our laboratory, the mice were housed singly in 28 x 13 x 17 cm. plastic cages provided with wood chip bedding and cotton nesting material. Wayne Mouse Breeder Blox (Allied Mills, Chicago) and water were available *ad libitum* before testing. The colony room was maintained on a 12:12 light:dark cycle, with lights off at 7:00 p.m. All subjects were ovariectomized under ether anesthesia at least 15 days before testing.

Apparatus

The olfactorium used in Study 2 was used in this study.

Experimental Design

A 2 x 2 x 2 x 2 Factorial Analysis of Variance was used for the major overall analysis of the data. The factors were: (a) species of the female *S* (*P. maniculatus* or *P. leucopus*); (b) species of the male mice providing the stimulus urine (*P. maniculatus* or *P. leucopus*); (c) the gonadal state of the female *S* (estrus or diestrus); and (d) the direction in the olfactorium from which the urine odor was presented (left or right). This latter condition was used to determine if a significant position preference occurred in the test apparatus. For convenience in setting up the analysis, the percent time spent away from the odor condition (i.e., the time with air) was used as the major dependent variable. For clarity in data presentation, however, the percent time spent with the odor in question was used. The latter is simply a linear transformation of the former. The number of crosses in the olfactorium during the test period was also examined.

Procedure

The general testing procedure of Study 2 was used in this experiment, with a few minor differences. First, in place of the 1 1/4 hr. test session of Study 2, each *S* was run for a single hour test session. Consecutive sessions usually began in the afternoon and often extended until midnight. As in Study 2, the olfactorium was thoroughly cleaned after each test. Second, estrus was artifically induced in the "estrous" Ss by daily 10 μg. estradiol benzoate/0.1 ml. sesame oil injections for four consecutive days, the last injection being followed 4 to 6 hours later by a single 500 μg. injection of progesterone in 0.1 ml. sesame oil. Diestrous mice were given the same sequence of injections of vehicle only. Vaginal smears were taken for each S before testing to insure the presence or absence of cornified epithelial cells indicative of estrus. While the side of odor presentation was systematically counterbalanced, the order of testing the Ss was initially randomized both within and between species. However, the availability of the animals did not always allow perfect randomization across the two species at all times, although a random sequence was possible in the majority of instances.

Male stimulus urine was collected from conspecific groups (2 to 4/group) of sexually-mature P. leucopus noveboracensis and P. maniculatus bairdi housed in plastic metabolism cages (Econo-cage Model 110, Maryland Plastics, Inc.). Urine was collected daily and added to separate 50 ml. graduated cylinders. No attempt was made to preserve the urine since old fermented urine has been suggested to be more attractive to rats than fresh urine (Müller-Velten, 1966; Reiff, 1956). Before each test, 1 to 2 ml. of urine was added from the appropriate graduated cylinder to a plexiglas collection box (the stimulus animal cage described in Study 1) and swished about to increase its contact with air. Separate collection boxes were used for the *P. maniculatus* and *P. leucopus* male urines. Fresh urine from the metabolism cage bottle was added to the graduated cylinders in a manner that resulted in its being at least 14 hours old when poured from the cylinders into the collection cage. The collection cages were not cleaned from the beginning of the experiment, allowing a cumulative build up of aging urine in a manner possibly analogous to what might happen to a spot urinated upon at intervals in a wild situation.

Only the data from the second half of the hour test session was subjected to Analysis of Variance, since Study 2 suggested that higher levels of activity during the early portions of the test session might confound the dependent variable. In order to eliminate *S*s who were in the middle of a circadian activity period or who froze and had little or no chance to sample both the odor and air situations, *S*s who made less than four crosses during the first half hour of the test or more than 200 total crosses during the entire test session were discarded.

Results

The mean percent times spent with the various odors, along with accompanying standard deviations, are presented for both the 1st and 2nd half hour periods in Table 6. Each value is based upon a total of eight Ss. The variances of the two half-hour groups did not depart significantly ($p > .10$) from homogenity ($F_{max}(8,7)$ values= 3.65 and 3.73 respectively). For comparative purposes, the median percent times spent with the various odors are presented for the two halves of the hour test period in Table 7.

Table 6. Mean percent time spent with male urine odor (vs. air) by estrous and diestrous female *P. maniculatus* and *P. leucopus* mice.

Species of Subject	Hormonal State	1st Half		2nd Half	
		$\bar{x}$	SD	$\bar{x}$	SD
Percent Time with *P. maniculatus* Odor					
P. maniculatus	Estrous	61.25	28.02	63.75	30.04
	Diestrous	59.38	34.44	67.38	39.61
P. leucopus	Estrous	57.63	23.79	50.63	35.87
	Diestrous	61.38	18.72	60.13	20.50
Percent Time with *P. leucopus* Odor					
P. maniculatus	Estrous	30.38	21.56	31.13	34.14
	Diestrous	47.75	32.32	30.75	27.57
P. leucopus	Estrous	51.75	34.20	58.13	39.23
	Diestrous	38.25	35.76	37.63	39.01

Table 7. Median percent time spent with male urine odor (vs. air) by estrous and diestrous female *P. maniculatus* and *P. leucopus* mice.

Species of Subject	Hormonal State	% Time with *P. maniculatus* odor		% Time with *P. leucopus* odor	
		1st Half Median	2nd Half Median	1st Half Median	2nd Half Median
P. maniculatus	Estrous	68.50	73.00	33.00	26.50
	Diestrous	63.50	88.50	34.50	29.00
P. leucopus	Estrous	56.50	35.50	48.50	62.50
	Diestrous	55.50	59.50	32.00	24.50

As evidenced by both the means and the medians, *P. maniculatus* females showed a strong tendency to spend more time with the urine odor of male *P. maniculatus* and less time with the urine odor of male *P. leucopus*, than with the air alternatives. Slight increases in both the mean and median amounts of time spent with the homospecific male odor occurred from the first to the second half-hour periods, regardless of the estrous condition. Decreases in the median amounts of time spent with *P. leucopus* male urine odor occurred across the two periods for both estrous and diestrous *P. maniculatus* females (Table 7). This effect was observed only between the two half-hour means of the diestrous *P. maniculatus* since the estrous means remained relatively constant (Table 6).

During the second half-hour period, estrous *P. leucopus* females showed a tendency to spend more mean and median time on the male *P. leucopus* odor side than on the air side. This effect was negligible during the first half hour (Tables 6 and 7). On the other hand, diestrous *P. leucopus* exhibited a strong tendency to avoid the urine odor of male conspecifics during both halves of the hour session. With the exception of the median percent time spent with the *P. maniculatus* odor during the second half hour period, both estrous and diestrous *P. leucopus* females showed a slight tendency to spend more time with male *P. maniculatus* odor than with air.

The means (± their standard errors) for the second half hour data for all experimental conditions are presented in Figure 8. These data were submitted to an overall 2 x 2 x 2 x 2 Factorial Analysis of Variance (Table 8). The major statistically significant effect from this analysis was that of odor condition (*P. maniculatus* male urine odor vs. *P. leucopus* male urine odor), although one three-way interaction, (odor x estrous state x odor direction) was also significant.

Although the species x odor interaction was not statistically significant ($.11>p>.10$), Figure 8 suggests the two species react quite differently to the odor conditions, and that an overall species x odor interaction may be supressed by relatively large variances and small sample sizes. To be conservative in accepting the conclusion that *P. maniculatus* male urine odors are preferred

Figure 8. Percent time spent with male urine odor (vs. air) by estrous and diestrous female *P. maniculatus* and *P. leucopus* mice during second half of hour test session.

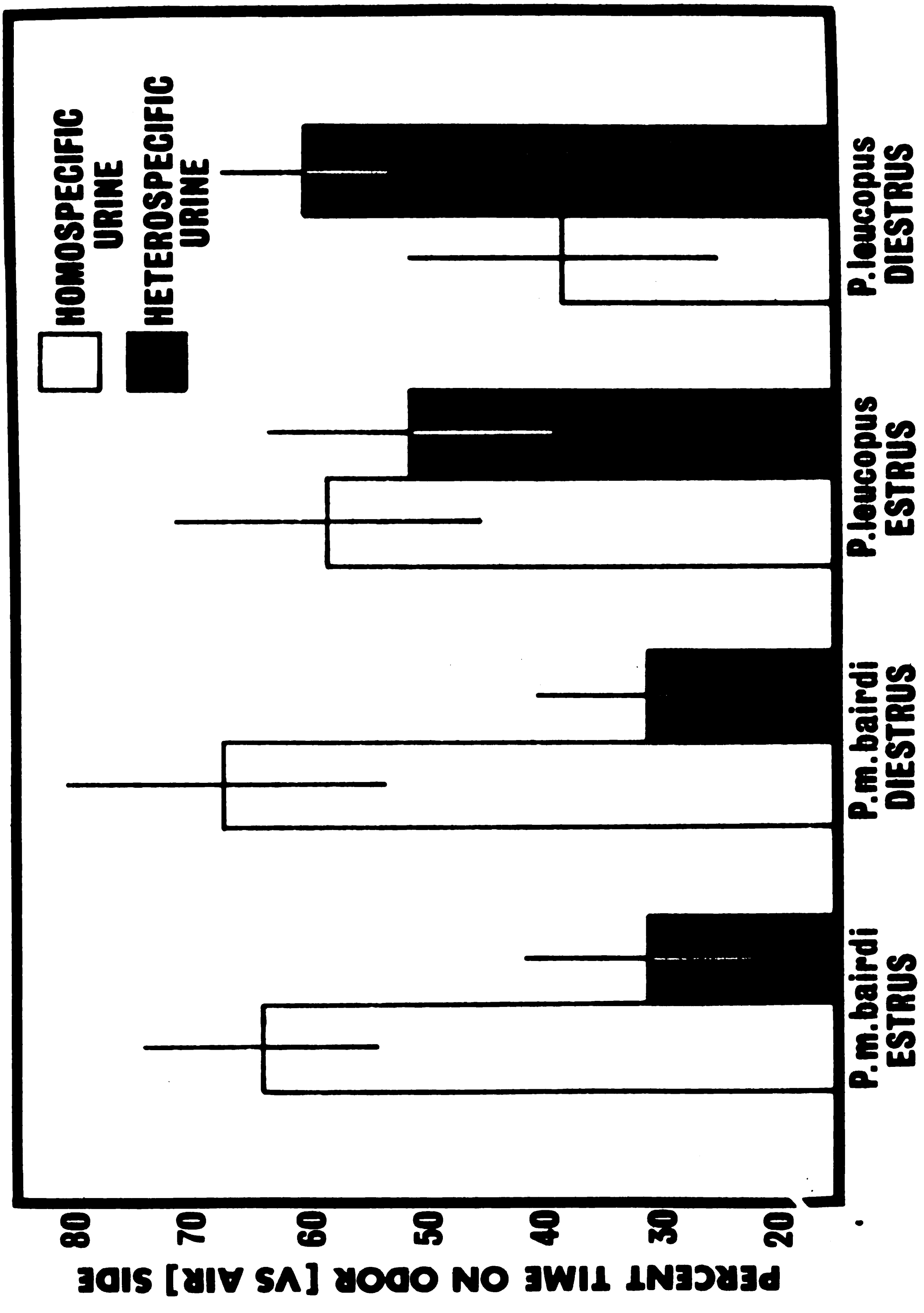

PERCENT TIME ON ODOR [VS AIR] SIDE
80
70
60
50
40
30
20
HOMOSPECIFIC URINE
HETEROSPECIFIC URINE
P.m.bairdi ESTRUS
P.m.bairdi DIESTRUS
P.leucopus ESTRUS
P.leucopus DIESTRUS

Table 8. Analysis of variance of female preferences for male urine odors during 2nd half of hour test session.

Source	DF	MS	F	P
Total	63			
Species of S	1	182.25	0.16	NS
Odor	1	7098.06	6.44	<0.02
Estrous State	1	60.06	0.05	NS
Odor Direction	1	30.25	0.03	NS
Species x Odor	1	2943.06	2.62	NS
Species x Estrous State	1	203.06	0.18	NS
Species x Odor Direction	1	420.25	0.37	NS
Odor x Estrous State	1	1156.00	1.03	NS
Odor x Odor Direction	1	742.56	0.66	NS
Estrous State x Odor Direction	1	351.56	0.31	NS
Species x Odor x Estrous State	1	676.00	0.60	NS
Species x Odor x Odor Direction	1	3220.56	2.86	NS
Species x Est. State x Odor Direction	1	5.06	0.00	NS
Odor x Estrous State x Odor Direction	1	4970.25	4.41	<0.04
Species x Odor x Estrous State x Odor Direction	1	380.25	0.34	NS
Error	48	1125.59		

more strongly than *P. leucopus* ones by both *P. leucopus* and *P. maniculatus* females, separate 2 x 2 Analyses of Variance (odor x estrous state) were performed for each species. *P. maniculatus* females spent significantly more time with male *P. maniculatus* urine odor (vs. air) than with male *P. leucopus* urine odor (vs. air) (Table 9, p< .025), whereas *P. leucopus* females did not (Table 10, p> .10).

Table 9. Analysis of variance of time spent with male urine odors by *P. maniculatus* females during 2nd half of hour test session.

Source	DF	MS	F	P
Total	31			
Estrous State	1	12.13	0.02	NS
Odor	1	9591.13	8.73	<0.025
Estrous State x Odor	1	32.00	0.03	NS
Within Cell	28	1099.13		

While these analyses do not negate the possibility that *P. leucopus* females prefer *P. maniculatus* male urine odor to air more than they prefer *P. leucopus* male urine odor to air, they do suggest that caution is warranted in making such a conclusion. Tables 6 and 7 suggest that such a conclusion may be warranted for diestrous, but not estrous, *P. leucopus* females.

Table 10. Analysis of variance of time spent with male urine odors by P. leucopus females during 2nd half of hour test session.

Source	DF	MS	F	P
Total	31			
Estrous State	1	242.00	0.20	NS
Odor	1	450.00	0.38	NS
Estrous State x Odor	1	1800.00	1.51	NS
Within Cell	28	1191.91		

As in Study 2, each S exhibited a consistent performance throughout the entire test session. Thus, the mean and median percent times with an odor during the first half hour were quite similar to those of the second half hour of the test sessions (Tables 6 and 7). Pearson product-moment correlations between the means of the first and second half-hour test sessions for each of the eight test conditions are presented in Table 11, and confirm this consistency.

To determine if attraction to the P. maniculatus odor was of a larger, smaller, or equivalent magnitude to the avoidance of the P. leucopus odor, the mean percent time spent with the P. maniculatus odor was compared to the mean percent time spent away from the P. leucopus odor (n= 32 in each group). The mean percent time spent with the P. maniculatus odor was nearly identical to the mean percent time spent away from the P. leucopus one (60.47% vs. 60.59%, SD = 31.36 & 35.41, respectively), suggesting that the tendency to approach the P. maniculatus odor was approximately equivalent to the tendency to avoid the P. leucopus one.

Table 11. Pearson product-moment correlations between 1st and 2nd half hour odor preferences.

Species and Gonadal State of Subject	ODOR TEST CONDITION	
	P. maniculatus (vs. air)	P. leucopus (vs. air)
P. maniculatus - estrus	0.89**	0.73*
P. maniculatus - diestrus	0.91*	0.85**
P. leucopus - estrus	0.94**	0.93**
P. leucopus - diestrus	0.93**	0.95**

**p< .005
*p< .025

A significant odor x estrous state x odor direction interaction was found in the overall analysis of variance (Table 8). This interaction is presented graphically in Figure 9. It is apparent that when the odor came from the left side of the olfactorium, both estrous and diestrous females showed a relative attraction to the P. maniculatus male urine, and a relative avoidance of the P. leucopus one, with estrous animals being slightly more extreme on both of these measures. On the other hand, when the odor

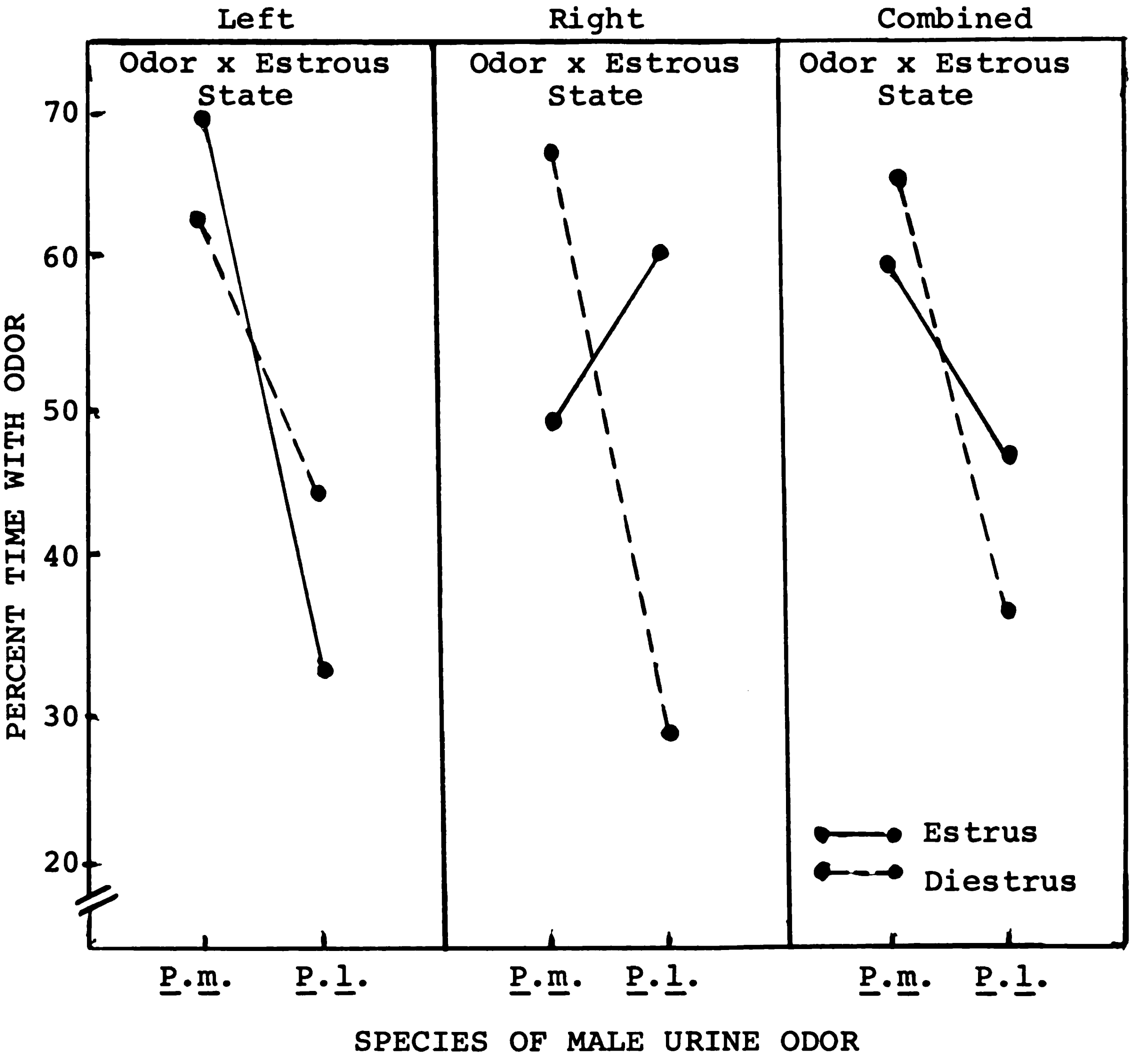


Figure 9. Geometric analysis of odor x estrous state x odor direction interaction.

came from the right, estrous _S_s showed a preference for the odor of _P. leucopus_ male urine, whereas diestrous ones did not.

The mean number of crosses in the olfactorium, along with accompanying standard deviations, are presented for the various experimental groups for the hour test sessions in Table 12. Regardless of estrous state, _P. leucopus_ females tended to be more active (in terms of crosses) in the presence of _P. maniculatus_ male urine odor than in the presence of _P. leucopus_ male urine odor. While diestrous _P. maniculatus_ showed a tendency to cross more while in the presence of _P. leucopus_ odor than while in the presence of _P. maniculatus_ odor, this was not true for estrous _P. maniculatus_. A 2 x 2 x 2 x 2 Factorial Analysis of Variance

Table 12. Mean number of crosses by estrous and diestrous female _P. maniculatus_ and _P. leucopus_ mice during hour test session.

Species of Subject	Hormonal State	# crosses with _P. maniculatus_ odor present		# crosses with _P. leucopus_ odor present	
		$\bar{x}$	SD	$\bar{x}$	SD
P. maniculatus	Estrus	57.25	37.99	50.00	40.96
	Diestrus	36.63	16.59	61.25	43.35
P. leucopus	Estrus	74.00	35.16	50.00	42.96
	Diestrus	112.25	53.07	57.25	51.94

on this data found a significant species difference and species x odor interaction (p< .03 and p<.02, respectively Table 13).

Table 13. Analysis of variance of number of olfactorium crosses by female _P. maniculatus_ and _P. leucopus_ during hour test session.

Source	DF	MS	F	P
Total	63			
Species of _S_	1	8395.14	4.95	<0.03
Odor	1	3407.64	2.01	NS
Estrous State	1	1080.77	0.64	NS
Odor Direction	1	102.52	0.06	NS
Species x Odor	1	9433.27	5.56	<0.02
Species x Estrous State	1	3094.14	1.83	NS
Species x Odor Direction	1	15.02	0.01	NS
Odor x Estrous State	1	2.64	0.00	NS
Odor x Odor Direction	1	4812.89	2.84	NS
Estrous State x Odor Direction	1	5058.77	2.98	NS
Species x Odor x Estrous State	1	4372.52	2.58	NS
Species x Odor x Odor Direction	1	2364.39	1.39	NS
Species x Estrous State x Odor Direction	1	722.27	0.43	NS
Odor x Estrous State x Odor Direction	1	1550.39	0.91	NS
Species x Odor x Estrous State x Odor Direction	1	2173.89	1.28	NS
Error	48	1695.11		

The median number of crosses per hour for the various experimental groups is presented in Table 14. The medians show the same directional relationships as the means (Table 12) and suggest, in some cases, slightly larger differences between the number of crosses of the heterospecific odor conditions and the number of crosses of the homospecific odor conditions than observed in the means.

Table 14. Median number of crosses by estrous and diestrous female *P. maniculatus* and *P. leucopus* mice during hour test session.

Species of Subject	Hormonal State	# Crosses with *P. maniculatus* odor present Median	# Crosses with *P. leucopus* odor present Median
P. maniculatus	Estrus	50.00	44.00
	Diestrus	35.50	58.50
P. leucopus	Estrus	85.00	51.00
	Diestrus	131.00	49.00

A Duncan's multiple range test (Winer, 1962) found that the mean number of crosses by the *P. leucopus* females tested with *P. maniculatus* odor was significantly greater than the mean number of crosses of each of the other groups, with the exception of the *P. leucopus* estrous one receiving *P. maniculatus* odor. None of the other comparisons were significant, although the difference between the mean number of crosses of the *P. leucopus* estrous group receiving *P. maniculatus* odor and the mean number of crosses of the *P. maniculatus* diestrous group receiving *P. maniculatus* odor approached significance ($p < .10$). The probability levels of the differences between the mean number of crosses of the *P. leucopus* diestrous animals receiving *P. maniculatus* odor and the other experimental groups are presented in Table 15.

Table 15. Probability levels for the differences between the mean number of crosses by *P. leucopus* diestrous females tested in the presence of *P. maniculatus* male urine odor and the mean number of crosses by mice in the other conditions of Study 3, using the Duncan's multiple range test.

Species	Hormonal State	Species of Male Urine Odor	P
P. maniculatus	Estrus	*P. maniculatus*	0.05
P. maniculatus	Diestrus	*P. maniculatus*	0.005
P. maniculatus	Estrus	*P. leucopus*	0.01
P. maniculatus	Diestrus	*P. leucopus*	0.05
P. leucopus	Estrus	*P. maniculatus*	NS
P. leucopus	Estrus	*P. leucopus*	0.05
P. leucopus	Diestrus	*P. leucopus*	0.05

To determine if the magnitude of odor preference or the number of crosses during a session was correlated with cumulative urine age for the eight experimental groups, Pearson product-moment correlations were computed. The majority of the correlations were small, and all were nonsignificant (Table 16). No systematic direction in the relations was apparent. Thus, under the present testing and urine collection procedures, no strong relationship was found between the age of the urine and the size of the _Ss_' odor preferences, or the number of crosses during the test session. The cumulative ages of urine used in this study ranged, in most cases, from 0 to 30 days.

Table 16. Pearson product-moment correlations between cumulative stimulus urine age and the dependent variables of Study 3.*

Subject Species and Estrous State	_P. maniculatus_ odor	_P. leucopus_ odor
r, urine age, % time with odor		
P. maniculatus estrous	0.27	-0.32
P. maniculatus diestrous	0.25	-0.09
P. leucopus estrous	-0.10	0.39
P. leucopus diestrous	0.11	-0.53
r, urine age, # crosses		
P. maniculatus estrous	-0.07	-0.16
P. maniculatus diestrous	-0.34	0.23
P. leucopus estrous	0.50	0.47
P. leucopus diestrous	0.06	-0.21

*All r's are nonsignificant, $p > .10$.

Discussion

The findings that the female *P. maniculatus* spent a relatively large proportion of the test time on the side of the olfactorium containing the odor of *P. maniculatus* male urine, and much less time on a side containing *P. leucopus* male urine odor, when these odors were paired with an air alternative, suggest the potential use of urine in helping to maintain sexual isolation between these two species. Although a careful examination of the use of urine by *P. leucopus* in marking has not been undertaken, *P. m. gambelii* are reported to use urine in marking their living areas by depositing it in corners and in areas of potential contact with strangers (e.g., in entrance ways between interconnected cages) (Eisenberg, 1962).

P. leucopus females also show a tendency to spend more time with *P. maniculatus* male urine odor than air, although this tendency is much less than that observed in the *P. maniculatus* females (Table 6, 7, and Figure 8). Bradshaw (1970) found, in a test situation similar to Smith's (1965) (see introduction for description), that female *P. m. nubiterrae* chose association with screened-off male conspecifics more often than with screened-off male *P. leucopus*. *P. leucopus* females were found to associate with male *P. m. nubiterrae* more often than with male conspecifics, although neither of these effects, independently, were significant. The findings of Study 3 are remarkably similar to the trends observed by Bradshaw (1970), and suggest that urine is a potential stimulus source mediating his study's observed preferences.

The effect of estrus observed in Studies 1 and 2 was not found in this study. A number of methodological differences between Study 3 and the previous ones do not make them directly comparable. First, only urine was used as the odor stimulus in Study 3, whereas urine, feces, and a live animal presumably producing sudiferous, sebaceous, and other types of odors were used in Studies 1 and 2. _P_. _m_. _bairdi_ males, for example, possess a midventral sebaceous glandular area, whereas _P_._l_. _noveboracensis_ males do not (Doty & Kart, 1972). Carr, Wylie & Loeb (1970) found that estrous, but not diestrous, sexually-naive female rats prefer the odor of intact to castrate males. Scott and Pfaff (1970), on the other hand, found that diestrous sexually-naive female _Mus musculus_ preferred the urine odor of intact males to that of castrates. The present data is more in line with that of Scott and Pfaff's study. Second, Study 3 used urine collected and pooled from a number of different males, whereas the urine and feces, as well as the other odor stimuli, came from only one male at a time in Studies 1 and 2. Third, the urine of Study 3 was presumably less fresh than some of the urine present in the stimulus configuration of the previous studies.

Fourth, the present study paired urine odor with air, whereas Studies 1 and 2 paired one odor complex with that of another. A heirarchy of odor preferences in rats obtained by giving a rat a choice between a lever producing a puff of air and one producing a puff of odorized air has been found to be much different from a heirarchy obtained from a paired-comparison two-lever situation (Tapp & Long, 1971). In fact, the results from these two methods correlated 0.02 (Schultz & Tapp, 1971). Differential rates of responding were used as the dependent variables by Tapp and Long (1971).

The high correlations obtained between the times spent on a given side of the olfactorium during the first and second halves of the hour test sessions (Table 11) are in agreement with the high reliability coefficients of Study 2 (Tables 4 and 5). Moore (1965) noted a similar consistency across time in a three-chamber test situation. This response stability may be an artifact of the mouse's "search strategy," rather than a product of the odor preference task, per se, since high intratest reliabilities have been obtained in a female P. m. bairdi tested without the presence of the experimental odors (Doty, personal observation).

The significant odor x estrous state x odor direction interaction (Table 8, Figure 9) suggests that estrous females react differently to the two types of male urine odors when the odor is presented from the right than when it is presented from the left of the olfactorium (Figure 8). Since the odor hoses were systematically counterbalanced within each experimental condition, this effect was presumably the result of some aspect of the olfactorium or testing room. No singularly compelling logical explanation of this effect is apparent to the present author.

In regards to activity (Table 12) the finding of a main effect of species (Table 13) may be misleading, since *P. leucopus* females were slightly less active in the presence of *P. leucopus* male odor than *P. maniculatus* females. On the other hand, in the presence of *P. maniculatus* male urine odor, *P. leucopus* females were much more active than *P. maniculatus* ones, which is demonstrated by the significant species x odor interaction (Table 13). While it is possible that the *P. leucopus* odor may have produced a decrease in the amount of crossing by *P. leucopus* females, it is also possible that the *P. maniculatus* odor produced an increase in the amount of crossing. Ropartz (1968a) reported that the odor of strange male conspecifics produced an increase in the activity of a group of male *Mus musculus*, with C57Bl/6 mice exhibiting the effect to a larger extent than C3H and Swiss strains. Urine (either fresh or fermented) was found to be the primary source of the odor producing this effect, although sudoriferous glands of the feet were also involved. Increased activity was not found in castrated animals, or in animals tested alone, suggesting some social behavior, such as aggression, may have produced the effect. Archer (1968) has subsequently demonstrated that strange male odor from soiled bedding increases the aggressiveness of *Mus musculus* males. The results of the present study suggest that crossing behavior, which typically subsides during an hour test, is higher in the presence of heterospecific male urine odor than in the presence of homospecific male urine odor in the two species tested.

Under the present test conditions, no strong and systematic relationship was discernable between the age of the cumulative stimulus urine build-up and either the percent time spent with a given odor or the number of crosses during the test period (Table 16). This finding does not, however, negate the possibility that urine age may be related to its stimulus value, since the present procedures produced a heterogeneously aged stimulus across time, as well as a presumably increasing stimulus quantity, which may confound the interpretation. Furthermore, besides the small number of subjects per group, the occasional exclusion of a mouse for failure to meet to crossing criteria (p. 78), and slight randomization differences in testing order between the experimental groups, limited careful evaluation of this factor.

In summary, Study 3 has demonstrated that male *P. maniculatus* urine odor is significantly more attractive than male *P. leucopus* urine odor to both estrous and diestrous P. maniculatus females, when these odors are paired with an air alternative. P. maniculatus male urine may also be more attractive to P. leucopus females, particularly diestrous ones, although caution is warranted in making this conclusion. P. leucopus male urine was found to elicit about as much avoidance as P. maniculatus male urine elicited attraction. The number of crosses in the olfactorium by P. leucopus females was found to be much greater in the presence of P. maniculatus male urine odor, than when in the presence of male P. leucopus urine odor. Age of cumulative urine deposition was not found to strongly correlate with either odor preference or crossing behavior, although this measure was confounded by several other factors. The urine odor preferences observed in this study were strikingly similar to preferences for nesting behavior found by Bradshaw (1970), suggesting that urine odor may be one stimulus factor capable of mediating spatial distribution preferences of P. maniculatus and P. leucopus females in a small testing area.

STUDY 4: HOMOSPECIFIC AND HETEROSPECIFIC FEMALE URINE ODOR PREFERENCES IN MALE *P. MANICULATUS* AND *P. LEUCOPUS*

While behavioral (e.g., Carr & Pender, 1958; Carr, Solberg, & Pfaffman, 1962) and neurophysiological (e.g., Pfaff & Pfaffman, 1969; Pfaff & Gregory, 1971) data suggest that normal and castrate male rats can distinguish between estrous and diestrous conspecific female urine odors, copulatory experience appears to be a prerequisite for the behavioral preference of estrous to diestrous female odors in this species (Carr, Loeb, & Dissinger, 1965). Although two studies have found that naive rats show such a preference (Le Magnen, 1952; Pranzarone, 1969), they have not been replicated by more carefully-controlled work (Carr, Loeb, & Dissinger, 1965; Stern, 1970). Copulatory experience has also been found to be necessary before early-androgenized female rats show a preference for estrous to diestrous female odor (Robertson & Whalen, 1970). Beach and Gilmore (1949) present data for dogs which suggest previous copulation may be necessary to produce a preference for estrous to diestrous urine odor, although other interpretations of their results can be made. Recent work with rats (Carr, Krames, & Costanzo, 1970) suggests that the nature of the previous copulatory experience is also of importance, for polygamous males with much sexual experience spent more time examining odors from novel than from familiar sex partners, whereas males with extensive monogamous sexual experience failed to show similar behavior.

The majority, if not all, of the studies examining male attraction to homo- and heterospecific females have used male subjects with unknown or poorly controlled histories of sexual experience (e.g., Godfrey, 1958; Moore, 1965; Smith, 1965). Furthermore, the only study found examining the male rat's behavior in relation to estrous and diestrous urine odors confounded sexual experience with the presence of testosterone (Pfaff & Pfaffman, 1969). Thus, little is known at present about the attractive or repulsive properties of female urine to homo- and heterospecific male rodents. The purpose of Study 4 was to determine the role of both estrous and diestrous aged female urine in attracting and/or repelling male *P. m. bairdi* and *P. l. noveboracensis* mice.

Method

Subjects

Thirty-two male *P. maniculatus bairdi* and 32 male *P. leucopus noveboracensis* served as subjects. At the time of testing the ages in days (mean ± S.D.) of these two groups were 96.00 ± 33.09 and 113.69 ± 43.23, respectively. The

_S_s were separated at weaning (21 days) into like-sexed conspecific groups of two to six animals, and housed in 28 x 13 x 13 cm. plastic cages containing wood chip bedding and nesting material. Purina mouse pellets and water were provided _ad libitum_. Following transportation from the Biology Research Center to our laboratory, the mice were housed singly in 28 x 13 x 17 cm. plastic cages provided with wood chip bedding and cotton nesting material. Wayne Mouse Breeder Blox (Allied Mills, Chicago) and water were available _ad libitum_. Some of the males were housed in a room with a reversed 12:12 light:dark cycle, with lights off at 7:00 a.m. The remainder were housed in the colony room, which was maintained on a 12:12 light:dark cycle, with lights off at 7:00 p.m.

Apparatus

The olfactorium used in Study 3 was used in this study.

Experimental Design

A 2 x 2 x 2 x 2 Factorial Analysis of Variance was used. The factors were: (a) species of the male _S_ (_P_. _maniculatus_ or _P_. _leucopus_); (b) species of the female mice providing the urine (_P_. _maniculatus_ or _P_. _leucopus_); (c) the gonadal state of the females providing the urine (estrus or diestrus); and (d) the direction in the olfactorium from which the urine odor was presented (left or right). The same dependent variables as used in Study 3 were used in this study.

Procedure

The general procedures of Study 3 were used. Urine was obtained from small groups of females: (a) diestrous *P. maniculatus bairdi*; (b) estrous *P. maniculatus bairdi*; (c) diestrous *P. leucopus noveboracensis*; and (d) estrous *P. leucopus noveboracensis*. All animals providing stimulus urine were ovariectomized at least 40 days prior to urine collection. Estrus was induced by the presence of a subcutaneously-implanted silastic capsule located in the dorsal neck region. These capsules were prepared by incorporating crystalline 17-Beta Estradiol Benzoate (EB) into a silicone rubber polymere (Dow Corning Medical Grade Silastic) and formed into solid tubes 5.5 mm. wide. These tubes were then cut at 3 mm. intervals, producing capsules with approximately 100 $mm.^2$ surface area, and containing (initially) approximately 1.7 mg. of EB apiece.[8] Cornette and Duncan (1970) demonstrated that a similar implant containing only 1 mg. EB kept a rat in persistent estrus until

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[8] I wish to thank Harold Hafs from the Dairy Science Department, Michigan State University, for his invaluable assistance in preparing these implants, and for furnishing both the silastic and crystalline hormone used for them.

the extraction of the implant at 251 days. In the present study, implants were used in place of injections because a number of problems resulted from taking the mice from the metabolism cages, injecting them, and replacing them. Furthermore, implants minimized the possibility of the excretion of an alarm substance into the urine following handling and injection procedures. All implanted mice of the present study showed persistent cornified vaginal smears during the period of urine collection.

Results

The mean percent times spent with the various odors, along with accompanying standard deviations, are presented for both the first and second half hours of the test period in Table 17. For comparative purposes, the medians of these periods are presented in Table 18.

During the second half of the test session, male *P. maniculatus* spent more relative time with estrous than diestrous *P. maniculatus* female urine odor, whereas *P. leucopus* males spent more time with diestrous than with estrous *P. maniculatus* odor, when air was the paired alternative. With the exception of the *P. maniculatus* tested with estrous heterospecific odor and the *P. leucopus* tested with diestrous homospecific odor, there was a tendency for the males to spend less time with the female urine odors during the second than during the first half-hour periods. The behavior of the males of both species in the presence of P. *leucopus* female urine odors, on the other hand, were variable across the half-hour periods, as evidenced by the shifts in the median times spent with the odor from proportions greater than 50 percent to less than 50 percent, and vice versa, in three of the four conditions (Table 18).

Table 17. Mean percent time spent with estrous and diestrous female urine odor (vs. air) by male P. *maniculatus* and P. *leucopus* mice.

Species of Subject	Hormonal State of Female Providing Urine	1st Half $\bar{x}$	1st Half SD	2nd Half $\bar{x}$	2nd Half SD
Percent Time with P. *maniculatus* Odor					
P. *maniculatus*	Estrus	57.75	11.76	49.38	12.62
	Diestrus	42.12	30.52	30.50	37.11
P. *leucopus*	Estrus	44.75	30.49	40.75	33.80
	Diestrus	62.25	24.95	57.87	33.74
Percent Time with P. *leucopus* Odor					
P. *maniculatus*	Estrus	55.00	20.81	61.00	19.12
	Diestrus	58.37	20.96	52.87	33.59
P. *leucopus*	Estrus	37.00	22.04	29.88	33.53
	Diestrus	34.25	35.95	53.87	41.62

The mean percent times spent with the various experimental odors (± standard errors) for the second half hour are

Table 18. Median percent time spent with estrous and diestrous female urine odor (vs. air) by male *P. maniculatus* and *P. leucopus* mice.

Species of Subject	Hormonal State of Female Providing Urine	% Time with *P. maniculatus* Odor		% Time with *P. leucopus* Odor	
		1st Half Median	2nd Half Median	1st Half Median	2nd Half Median
P. maniculatus	Estrus	66.00	41.50	49.50	65.50
	Diestrus	44.00	9.00	63.50	44.50
P. leucopus	Estrus	32.00	28.00	35.50	30.00
	Diestrus	69.00	63.00	18.50	62.00

presented in Figure 10. The variances of these groups did not differ from homogeneity ($F_{max}(8,7) = 10.88$). These data suggest a potential interaction between species and estrous state, since *P. maniculatus* males spent more time with estrous relative to diestrous odors, and *P. leucopus* males spent more time with diestrous relative to estrous ones, when the odors were paired with air alternatives. The results of a 2 x 2 x 2 x 2 Factorial Analysis of Variance on these data (Table 19) confirmed the statistical presence of this interaction ($p< .04$), and also indicated the presence of a very strong position preference by the males for the right side of the olfactorium. This preference was independent of odor condition, and accounted for nearly one quarter of the total variance.

Figure 10. Percent time spent with female urine odor (vs. air) by male *P. maniculatus* and *P. leucopus* mice.

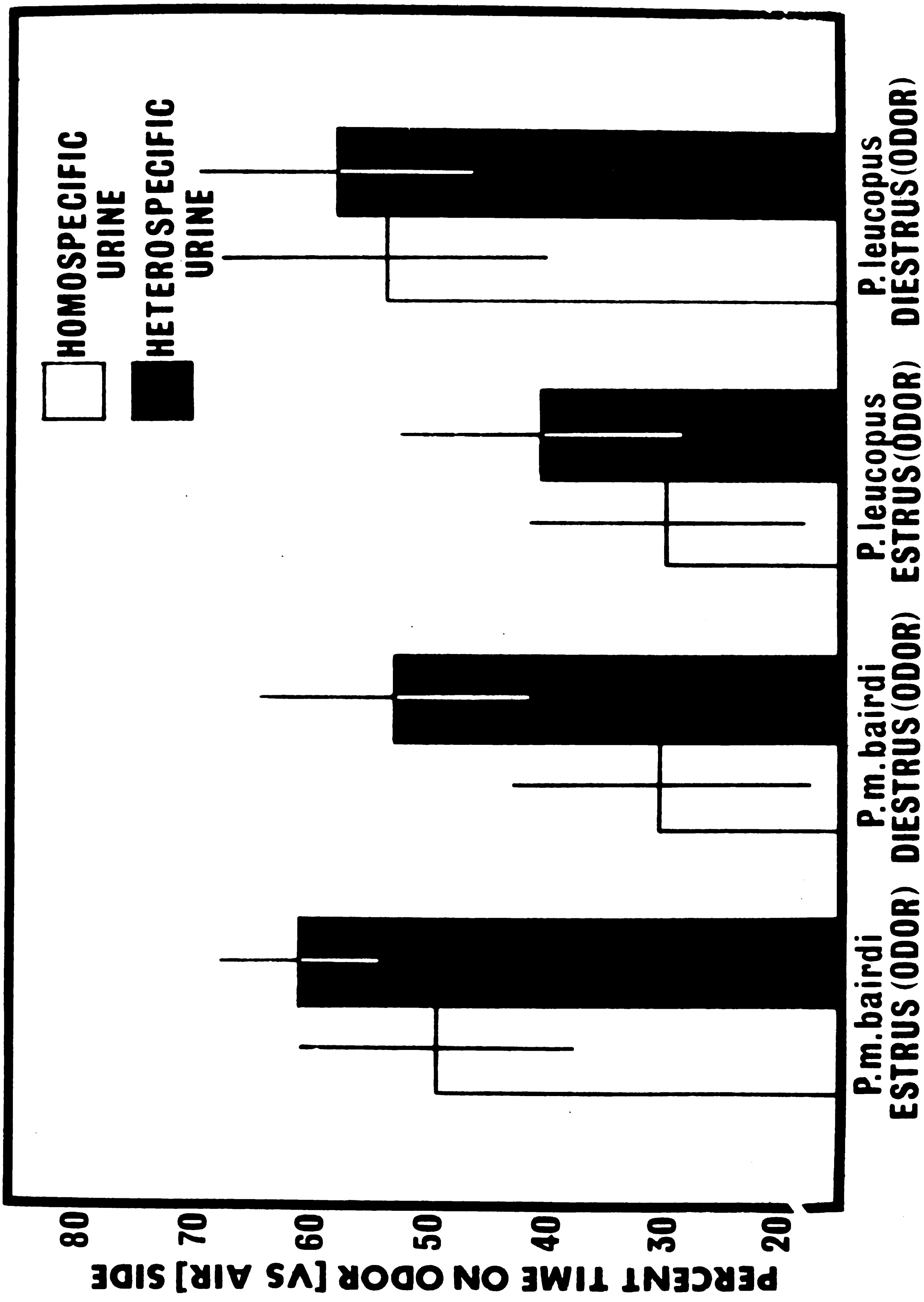
PERCENT TIME ON ODOR [VS AIR] SIDE
20
30
40
50
60
70
80
HOMOSPECIFIC URINE
HETEROSPECIFIC URINE
P.m.bairdi ESTRUS (ODOR)
P.m.bairdi DIESTRUS (ODOR)
P.leucopus ESTRUS (ODOR)
P.leucopus DIESTRUS (ODOR)

Table 19. Analysis of variance of male preferences for female urine odors during 2nd half of hour test session.

Source	DF	MS	F	P
Total	63			
Species of S	1	150.06	0.16	NS
Odor	1	400.00	0.44	NS
Estrous State of Female Providing Urine	1	225.00	0.25	NS
Odor Direction	1	18837.56	20.55	<0.0005
Species x Odor	1	2475.06	2.70	NS
Species x Estrous State	1	4522.56	4.93	<0.04
Species x Odor Direction	1	0.00	0.00	NS
Odor x Estrous State	1	342.25	0.37	NS
Odor x Odor Direction	1	39.06	0.04	NS
Estrous State x Odor Direction	1	126.56	0.14	NS
Species x Odor x Estrous State	1	22.56	0.02	NS
Species x Odor x Odor Direction	1	484.00	0.53	NS
Species x Estrous State x Odor Direction	1	400.00	0.44	NS
Odor x Estrous State x Odor Direction	1	826.56	0.90	NS
Species x Odor x Estrous State x Odor Direction	1	12.25	0.01	NS
Error	48	916.66		

A simple effects analysis for the species x estrous state interaction (Winer, 1962) was performed by examining the data from each species by separate 2 x 2 (odor x estrous state) Analyses of Variance. Neither of these analyses found a significant effect due to the estrous condition of the female producing the urine (Tables 20 and 21).

Table 20. Analysis of variance for time spent with female urine odors by *P. maniculatus* males during second half of hour test session.

Source	DF	MS	F	P
Total	31			
Odor	1	2178.00	2.15	NS
Estrous State of Female Providing Urine	1	1352.00	1.33	NS
Odor x Estrous State	1	275.66	0.27	NS
Within Cell	28	1013.60		

Although not statistically significant, a slight tendency of the males of both species to spend more time with the heterospecific than the homospecific odor, when each was paired with an air alternative, was present in this study. (Tables 17 and 18).

As in Studies 2 and 3 (Tables 4, 5, and 11), each subject showed reliable response patterns, as indicated by high Pearson product-moment correlations between the percent

Table 21. Analysis of variance for time spent with female urine odors by P. leucopus males during second half of hour test session.

Source	DF	MS	F	P
Total	31			
Odor	1	442.53	0.34	NS
Estrous State of Female Providing Urine	1	3382.53	2.63	NS
Odor x Estrous State	1	94.53	0.07	NS
Within Cell	28	1284.58		

time spent on a given side during the first and second halves of the hour test session (Table 22). The only nonsignificant correlation was found in the P. maniculatus males receiving estrous P. leucopus female urine odor. The magnitudes of the

Table 22. Pearson product-moment correlations between 1st and 2nd half hour odor preferences.

Species of Subject	Gonadal State of Female Mouse Providing Urine	ODOR TEST CONDITION	
		P. maniculatus (vs. air)	P. leucopus (vs. air)
P. maniculatus	Estrous	0.86**	-0.14
P. maniculatus	Diestrous	0.81**	0.93**
P. leucopus	Estrous	0.99**	0.96**
P. leucopus	Diestrous	0.87**	0.65*

**p< .005
*p< .05

mean or median percent times spent with the urine odor for each of the two halves of the test session were quite similar for these animals (Tables 17 and 18), although they did not show the characteristics decline in odor preference from the first to second half of the test period.

The mean number of crosses (along with accompanying standard deviations) in the olfactorium during the entire hour test session is presented for the various experimental groups in Table 23. Table 25 presents the results of a 2 x 2 x 2 x 2 Analysis of Variance on these data. The only statistically significant source of variance was the species x odor x odor direction interaction ($p < .05$, Table 25). This interaction is presented graphically in Figure 11. The median number of crosses (Table 24) suggests a general tendency for more crosses to occur in the presence of heterospecific than in the presence of homospecific female urine odor,

Table 23. Mean number of crosses by male _P. maniculatus_ and _P. leucopus_ mice during hour test session.

Species of Subject	Hormonal State of Female Mouse Providing Urine	# Crosses with _P. maniculatus_ odor present		# Crosses with _P. leucopus_ odor present	
		$\bar{x}$	SD	$\bar{x}$	SD
P. maniculatus	Estrous	57.50	36.39	99.25	56.89
	Diestrous	74.38	70.01	63.25	44.23
P. leucopus	Estrous	63.00	54.00	64.38	50.23
	Diestrous	69.38	31.01	42.25	28.42

just as did the mean number of crosses (Table 23). Only the *P. leucopus* males tested with estrous odors failed to exhibit this effect. However, when the data were collapsed across species and estrous groups into homospecific-odor and heterospecific-odor categories, the median number of crosses of the heterospecific-odor group (median = 63.50) was not significantly greater than that of the homospecific-odor one (median = 52) (Mann-Whitney U (32,32) = 411.5, p>.10).

Table 24. Median number of crosses by male *P. maniculatus* and *P. leucopus* mice during hour test session.

Species of Subject	Hormonal State of Female Mouse Providing Urine	# Crosses with *P. maniculatus* odor present Median	# Crosses with *P. leucopus* odor present Median
P. maniculatus	Estrous	43.50	93.50
	Diestrous	39.00	54.50
P. leucopus	Estrous	56.25	46.00
	Diestrous	67.00	41.00

As in Study 3, Pearson product-moment correlations were computed between both the number of crosses and the mean percent times spent with a given odor, and the cumulative urine age for each of the eight experimental groups (Table 26). With the exception of the correlation between the cumulative diestrous *P. maniculatus* female urine age and the number of crosses by *P. leucopus* males (r = 0.79,

Table 25. Analysis of variance of male crosses during hour test session.

Source	DF	MS	F	P
Total	63			
Species of S	1	2983.89	1.33	NS
Odor	1	31.64	0.01	NS
Estrous State of Female Providing Urine	1	1269.14	0.57	NS
Odor Direction	1	3921.89	1.75	NS
Species x Odor	1	3094.14	1.38	NS
Species x Estrous State	1	6.89	0.00	NS
Species x Odor Direction	1	252.02	0.11	NS
Odor x Estrous State	1	6744.52	3.00	NS
Odor x Odor Direction	1	6.89	0.00	NS
Estrous State x Odor Direction	1	3555.14	1.58	NS
Species x Odor x Estrous State	1	558.14	0.25	NS
Species x Odor x Odor Direction	1	9925.14	4.42	<0.05
Species x Estrous State x Odor Direction	1	2150.64	0.96	NS
Odor x Estrous State x Odor Direction	1	1881.39	0.84	NS
Species x Odor x Estrous State x Odor Direction	1	284.77	0.13	NS
Error	48	2246.23		

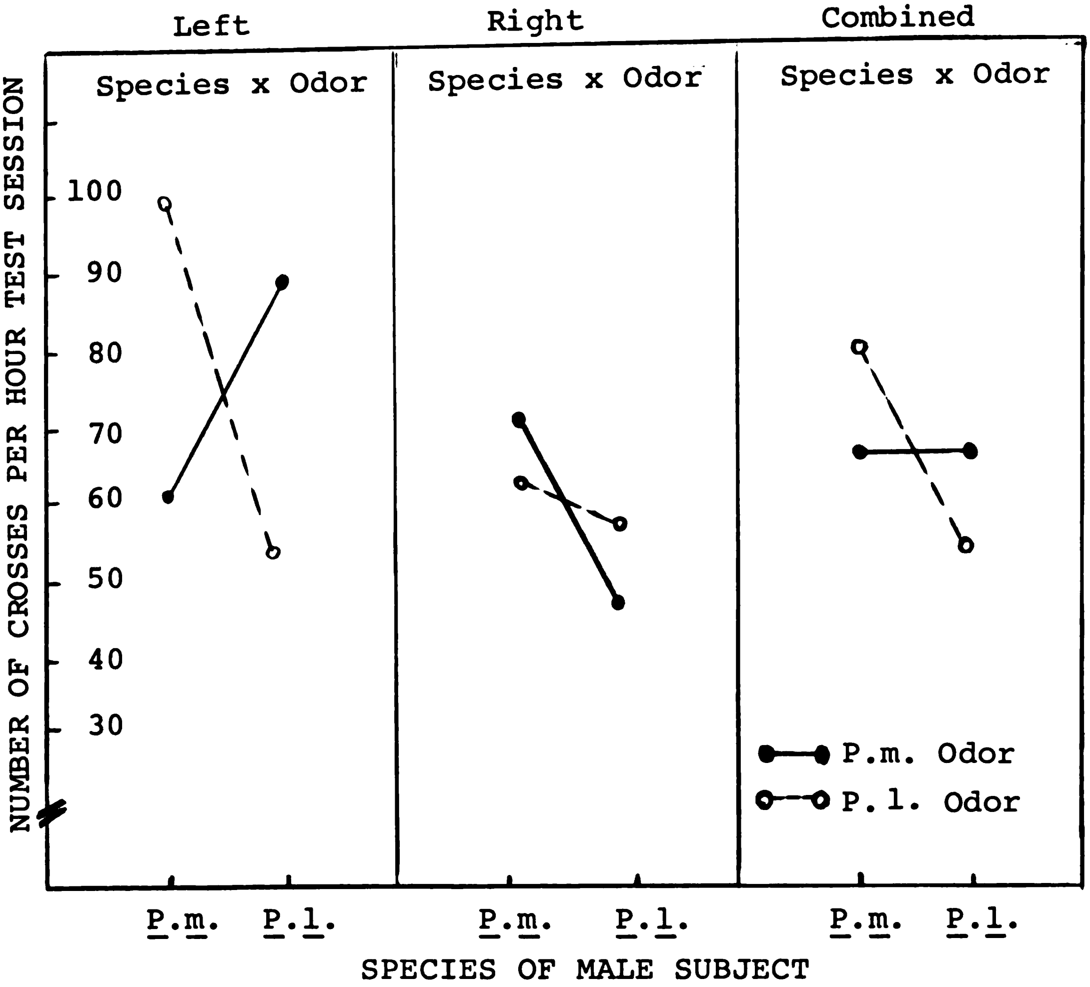

Figure 11. Species x odor x odor direction interaction for the number of crosses by subjects of Study 4.

$p < .02$), all correlations were relatively small and statistically nonsignificant.

Table 26. Pearson product-moment correlations between cumulative stimulus urine age and the dependent variables of Study 4.

Subject Species and Estrous State of Female Mice Providing Stimulus Urine	_P. maniculatus_ odor	_P. leucopus_ odor
	r, urine age, % time with odor	
P. maniculatus estrous	-0.35	-0.17
P. maniculatus diestrous	-0.34	0.14
P. leucopus estrous	-0.09	0.43
P. leucopus diestrous	-0.50	0.11
	r, urine age, # crosses	
P. maniculatus estrous	-0.57	-0.12
P. maniculatus diestrous	0.31	-0.53
P. leucopus estrous	0.08	0.40
P. leucopus diestrous	0.79*	-0.11

*$p < .02$.

Discussion

A major (and unexpected) finding of Study 4 was a marked position preference by the males for the right side of the olfactorium, which was relatively independent of the odor condition. This preference may have been mediated by either intraolfactorium (e.g., ultrasonic sound differences from the two whisper fans) or extraolfactorium (e.g., light gradient at the time of initial placement into the olfactorium) cues. In a current study, the olfactorium has been rotated 180 degrees in order to determine the relative importance of these two sources. If intraolfactorium cues are the more important, a preference for the left side of the olfactorium should emerge. On the other hand, if extraolfactorium cues are most important, the preference for the right should prevail. Current data suggest the latter to be the more salient of the two sources. One possible extraolfactorium explanation suggests that sex differences exist in relation to escape strategies related to noxious stimulation (the females of Study 3 did not exhibit a strong position preference). The right side of the olfactorium was distally related to both the hallway door through which the mouse had been carried by the tail with tongs, and the experimenter's general body position (the experimenter was right handed, resulting in the placement of his body slightly to the left of center of the olfactorium). An escape from these two sources of cues associated with noxious stimulation (e.g., handling with the tongs) mediated primarily in terms of spatial contingencies would result in the preference for the right side of the olfactorium. It appears probable that the males did not rely heavily upon olfactory cues in determining the initial direction of avoidance. It is theoretically possible that the presence or absence of odor served as a discriminative stimulus for a "safe" area (e.g., one associated with reduction of fear), and thereby helped to maintain a position habit throughout the test session. A number of studies suggest that male rodents are more "emotional" or "fearful" than female ones in various test situations. For example, open field defecation has been observed to be higher in male rats than in female rats (e.g., Anderson, 1940; Broadhurst, 1957, 1958; Denenberg & Morton, 1962). Female rats ambulate more in such a situation (e.g., Broadhurst, 1957, 1958; Denenberg & Morton, 1962; Sines, 1961) and tend to emerge more quickly in tests of "timidity" (emergence tests) (e.g., Anderson, 1940; Meyers, 1962). Female rats have been observed to explore novel territory more than males (Richards & Leslie, 1962; Thompson, 1953; Zimbardo & Montgomery, 1957), and learn active avoidance responses more quickly than males (e.g., Beatty & Beatty, 1970; Levine & Broadhurst, 1963; Nakamura & Anderson, 1962). These well-documented sex differences in relation to strange, novel, and noxious situations may, in part, help to account for the position preference differences observed between the males of Study 4 and the females of Study 3. Of course, more data are needed to determine the relative importance of these and other conceivable factors in producing the present differences.

The tendency for *P. maniculatus* males to spend more time with estrous relative to diestrous female urine odors, along with the tendency for *P. leucopus* males to spend more time with diestrous than with estrous female urine odors, when all odors are paired with an air alternative (Species x Estrous interaction, Table 19), should be conservatively interpreted. During the first half hour (Table 17), *P. maniculatus* males spent slightly more time with diestrous than with estrous odors, whereas *P. leucopus* males spent more time with estrous relative to diestrous *P. leucopus* female urine odors. Furthermore, when the data for each species is analyzed by separate Analyses of Variance (Tables 20 and 21), no significant effects due to the estrous condition of the females producing the urine were found. On the other hand, these odor preference differences may be valid, since *P. leucopus* are known to generally nest alone during the breeding season, which might be mediated, in part, by avoidance of urine odors associated with a sexually-active female's nest box. *P. maniculatus*, on the other hand, are often found nesting together during the breeding season (Nicholson, 1941).

The failure to find a clear-cut preference for estrous over diestrous urine odor may be due to a number of potentially interacting factors. First, since the mice of the present study were sexually-naive, they may not exhibit a preference for estrous to diestrous female urine odor.

Several studies suggest that sexual experience may be a necessary prerequisite for such a preference in some rodents (Carr, Loeb, & Dissinger, 1965; Robertson & Whalen, 1970; Stern, 1970). Second, since the urine of this study was not fresh, an odor due to estrus may have dissipated by the time of testing. If estrous state is to be reliably communicated in wild situations, such a breakdown of products would appear *prima facie* to facilitate the communication of accurate information. Third, the present method of inducing estrus in the females did not include the use of progesterone, which theoretically could influence the odor of the urine. In spayed female housemice, exogenously administered estrogen and progesterone given in sequence are more effective than estrogen alone in inducing sexual receptivity (Ring, 1944). Finally, it is possible that urine is not a stimulus source mediating the communication of estrous state in the species examined in this study.

As in Studies 2 and 3, the mice of Study 4 generally responded consistently throughout the hour test session, as indicated by high intratest reliability coefficients (Table 22). The single low correlation ($r = -0.14$, *P. maniculatus* male subjects, *P. leucopus* estrous odor) was due, in part, to large preference shifts between the two halves of the test session by four of the subjects (48% to 75%, 97% to

38%, 43% to 67%, and 39% to 58%). The remaining males of this group exhibited consistent responses across the two half-hour periods.

An examination of the species x odor x odor direction interaction for the number of crosses in the olfactorium reveals that when the odor came from the left, the mice were more active in the presence of heterospecific than in the presence of homospecific urine odor (Figure 11). On the other hand, when the odor came from the right, the males were slightly more active in the presence of homospecific urine odor than in the presence of heterospecific urine odor. A clear-cut reason for this difference is not readily apparent to the present author. However, one (of many) hypothetical explanation for these results is as follows: A strange (heterospecific) odor's presence on the side opposite a safe side (one associated with escape from noxious stimulation, as previously suggested in this discussion section) might result in a relatively short exploration of that side, with the mouse returning to the safe side or "homebase" after smelling the strange odor. However, with a constant activity or exploratory drive level, the mouse would soon again cross to the side containing the strange odor, only to retreat in short order. These alternations would tend to result in a high overall crossing level, therefore, when the heterospecific odor came from the left (Figure 11). On the other hand, when an odor came from the right, it became associated with the safe or home-base area. Crosses into the opposite or nonodor side would not result in an immediate return to the safe side, so that the overall level of activity or crossing would not be very high. Furthermore, the presence of homospecific odor on the safe side might reassure the mouse and possibly lessen its fear, thereby facilitating the exploration of the opposite side, and helping to produce a few more exploratory attempts. While this post hoc explanation may be useful in helping to generate hypotheses for future work, it is one of many explanations which could be called forth to explain these results, and should not be taken too seriously without further data to establish its validity.

As in Study 3, Pearson product-moment correlations between the cumulative urine age and both the number of crosses in a session and the percent time with a given odor were generally small, nonsignificant, and variable in direction (Table 26). A 0.79 ($p < .02$) correlation between the cumulative _P. maniculatus_ diestrous urine age and the number of crosses by _P. leucopus_ males was found. Although this may be a true correlation, it is also possible that this correlation is due to chance, since out of the 32 correlations calculated for the cumulative urine age and the dependent variables of Studies 3 and 4 (Tables 16 and 26), at least one would be expected to be significant by chance at the .02 level.

GENERAL CONCLUSIONS AND SOME SELECTED COMPARISONS BETWEEN MALE AND FEMALE RESPONSES TO URINE ODORS

In the Introduction, a review of the rodent olfactory literature was undertaken, and a hypothesis was formulated suggesting that courtship phenomena similar to those observed in a number of birds, fish, and amphibia are likely present in some species of rodents. Olfaction, instead of audition or vision, was suggested to play a major role in relation to these behaviors. Support for the notion of highly-odored males and cryptically-odored females (analogous to highly-colored males and cryptically-colored female birds) was obtained from morphological studies evidencing large odor producing glands in males which were either absent or greatly reduced in females (e.g., Doty & Kart, 1972; Mykytowycz, 1970). Androgen dependent sebaceous glands were shown to be inhibited by estrogen, either alone (Hooker & Pfeiffer, 1943) or in combination with progesterone (Doty & Kart, 1972), and have been implicated in effecting the odoriferous properities of both urine (Bronson & Caroom, 1971) and feces (Hesterman & Mykytowycz, 1968) in some species of rodents and lagomorphs. The important role of male odors in effecting a number of female endocrinological processes was reviewed (e.g., Bruce, 1959, 1960; Bruce & Parrott, 1960; Whitten, 1956a, 1956b; Vandenberg, 1967, 1969) and data from a rodent behavioral study was examined which suggested that female *Mus musculus* are attracted to conspecific male sebaceous glandular products produced by the preputial gland (Bronson & Caroom, 1971) and deposited in the urine during micturation. A number of studies were reviewed which suggested that a urinary substance, not necessarily dependent upon preputial secretions, elicited aggressive responses on the part of male conspecific *Mus musculus* (e.g., Archer, 1968; Mackintosh & Grant, 1966; Mugford & Nowell, 1970, 1971b). A determination of whether male urine odors are avoided by *P. maniculatus* and *P. leucopus* males is currently being made by the present author. If avoidance occurs, then it would appear that two major functions of courtship display by male birds--namely, attraction of females and the warding off of males--may be served by urinary odors in mice. A number of studies were reviewed which suggested that some female rodents may be more sensitive to early odor experiences than males, as evidenced by preference behaviors in later life (e.g., Carter, 1969; Mainardi, Marsan & Pasquali, 1965; Quadagno & Banks, 1970). Behavioral evidence was cited suggesting that open area movements by both female mice (*Mus musculus*) (Mackintosh, 1970) and gerbils (Thiessen, Blum, & Lindsey, 1970) are little influenced by territorial boundaries or the presence of sebaceous sebum in the testing situation, whereas the movements of males are restricted by such factors. In *P. maniculatus*, one study presented evidence suggesting the female selection of a male nesting partner and the movement of the female into a male's home area (Hill, 1970). A review of a number of studies suggested that both sexes of several species of mice can distinguish between their own and different species on the basis of olfactory cues alone (e.g., Bowers & Alexander, 1967; Moore, 1965; Smith, 1965). The primary purpose of this section is to compare some of the findings of Study 3 with some of those of Study 4 in an effort to determine if the female is more responsive to opposite-sexed urinary odors than the male, and if these differences might potentially be related to sexual isolation of the two species under consideration. Some general conclusions and speculations in regards to the data from the present series of studies will also be presented.

The data of Tables 27 and 28 (compiled from Tables 6, 7, 17, and 18) allow a comparison of both the mean and median times spent with opposite-sex urine odor (vs. air) by the male (Study 4) and female (Study 3) subjects. In Table 23, for example, a comparison can be made between (a) the average amount of time estrous female *P. maniculatus* spent with the odor from male *P. maniculatus* urine (vs. air) and (b) the average amount of time male *P. maniculatus* spent with the urine odor from estrous *P. maniculatus*.

Table 27. Mean percent times spent with opposite-sex urine odor by male and female *P. maniculatus* and *P. leucopus* during second half of test session--data from Tables 6 and 17.

Species of Subject	Estrous State of Female subject or of females providing stimulus urine	Female *S*s	Male *S*s	Diff.
% Time spent with homospecific opposite sex urine odor				
P. maniculatus	Estrous	63.75	49.38	14.37
	Diestrous	67.38	30.50	36.88**
P. leucopus	Estrous	58.13	29.88	28.25*
	Diestrous	37.63	53.87	16.24
% Time spent with heterospecific opposite sex urine odor				
P. maniculatus	Estrous	31.13	61.00	29.87**
	Diestrous	30.75	52.87	22.12
P. leucopus	Estrous	50.63	40.75	9.88
	Diestrous	60.13	57.87	2.26

$^{**}p < .025$ One-tailed t-tests
$^{*}p < .05$

It is apparent from both Table 27 and Table 28 that the female *P. maniculatus* exhibited a stronger relative attraction to the homospecific opposite-sex urine odor than did the male *P. maniculatus*, regardless of the estrous factors involved. Furthermore, female *P. maniculatus* showed a stronger avoidance of opposite-sex heterospecific urine than did males, also regardless of the estrous conditions. Statistically significant differences occurred, however,

Table 28. Median percent times spent with opposite-sex urine odor by male and female P. maniculatus and P. leucopus during second half of test session--data from Tables 7 and 18.

Species of Subject	Estrous State of female subject or of females providing stimulus urine	Female Ss	Male Ss	Diff.
% Time spent with homospecific opposite sex urine odor				
P. maniculatus	Estrous	73.00	41.50	31.50
	Diestrous	88.50	9.00	79.50*
P. leucopus	Estrous	62.50	30.00	32.50
	Diestrous	24.50	62.00	37.50
% Time spent with heterospecific opposite sex urine odor				
P. maniculatus	Estrous	26.50	65.50	39.00*
	Diestrous	29.00	44.50	15.50
P. leucopus	Estrous	35.50	28.00	7.50
	Diestrous	59.50	63.00	3.50

*p< .025 One-tailed Mann-Whitney U-tests

only in the diestrous homospecific odor condition (t (14) = 2.27, p< .025; U (8, 8) = 12.5, p< .025) and the estrous heterospecific odor condition (t (14) = 2.16, p< .025; U (8, 8) = 13, p< .025). The results for P. leucopus, on the other hand, are less systematic. Estrous female P. leucopus spent significantly more mean time with opposite-sex homospecific urine odor than did male P. leucopus (t (14) = 1.92, p< .05), but the differences between the medians of the females and males was not significant (U (8,8) = 28, p>.10).

Furthermore, the absolute differences between the male and female *P. leucopus* mean preferences were relatively small in the remaining conditions, and all were smaller than the differences observed in comparable conditions for the *P. maniculatus* (16.24 vs. 36.88; 2.26 vs. 22.12; and 9.88 vs. 29.87). An examination of possible sex differences in the first half-hour means (Tables 6 and 12) discloses the same direction of differences for all the odor conditions in *P. maniculatus* subjects, as well as for the subjects in the *P. leucopus* estrous condition. In the remaining *P. leucopus* conditions, very small reversals occurred. However, even during the second half hour sessions (Table 27), only very small mean sex differences are present in these conditions.

The present data support the notion that female *P. maniculatus* react more strongly than male *P. maniculatus* to opposite-sex urine odors in directions that could conceivably result in a higher probability of encounters with conspecific males and a lower probability of encounters with heterospecific males. Thus, it is possible that sexual isolation between *P. maniculatus* and *P. leucopus* may be maintained, in part, by the behaviors of the *P. maniculatus* females to urinary odors. The evidence for *P. leucopus* females, on the other hand, is much more equivocal, since the mean sex differences were quite small in three of the four conditions (Table 27), and since *P. leucopus* females show, in some instances, slight preferences for male *P. maniculatus* urine odor to air (Figure 8, Study 3). A significant sex difference found for *P. leucopus* in regards to homospecific urine odor when the estrous factor was involved may be primarily the result of the strong avoidance of the female urine odor by the males (29.88%), since the mean homospecific attraction by the *P. leucopus* females was quite low (58.13%) compared to the homospecific attraction exhibited by the estrous and diestrous female *P. maniculatus* (63.75% and 67.38%, respectively). Thus, the potential use of urine in helping to maintain sexual isolation appears to be less likely in *P. leucopus* than in *P. maniculatus*.

While the data are limited, there is suggestive evidence of marked differences between *P. maniculatus bairdi* and *P. leucopus noveboracensis* in terms of their potential social organization and reliance upon a variety of olfactory cues. Hill (1970) has shown that these two species "differ greatly in their mutual nesting habits (p. 48)." In a four-compartment test situation, *P. maniculatus*, but not *P. leucopus*, exhibited a statistically significant pattern of the female moving in with a nearby male. Furthermore, heterosexual pairs of *P. maniculatus* were found together on a significantly greater number of days than were homosexual pairs of *P. maniculatus*, or homo- and heterosexual pairs of *P. leucopus*. These differences suggest to the present author that pair-bonding may be more prevalent in *P. maniculatus* than in *P. leucopus*, and that the contentions of the Introduction may be more likely to apply to P. maniculatus than to P. leucopus. Nicholson's (1941) data support the notion of promiscuity by male Peromyscus leucopus. The data from Studies 3 and 4 are generally more clear-cut (in terms of expected directions and magnitudes of preferences) for P. maniculatus than for P. leucopus, suggesting the potentially more reliable use of urine odors in attraction and avoidance behaviors by this species. Doty and Kart (1972) present evidence that P. maniculatus bairdi possess a large midventral sebaceous glandular area which is possibly used for intraspecific communication in wild situations, whereas P. leucopus noveboracensis do not, suggesting at least one major anatomical difference in the odor-producing capability between the two species. Clemens (1969) found that males of another maniculatus subspecies, P. m. gambeli, had a much shorter latency to mount females when the female had previously copulated with another male than when the female was fresh. While elicitation behavior on the part of the female cannot be ruled out, olfaction might be involved in the effect, suggesting that males can use olfactory cues at close range for consummatory behavior. A similar phenomenon has not been reported, to the author's knowledge, in any other species of mammal.

Since P. maniculatus are generally found in open habitats, while P. leucopus inhabit primarily upland mixed hardwood forests of hickory and oak (Nicholson, 1941), differential reliance upon sensory modalities might be expected. *P. leucopus noveboracensis* have heavier eye lenses in relation to body weight than *P. maniculatus bairdi* (King, 1968), although the functional significance of this difference is not clear. The visual acuity (obtained from optokinetic nystagmus tests) of both species has been found to be keener than that of any other rodent species tested to date (Vestal, 1970). However, a small visual angle does not necessarily imply a correlation with function or use of the modality in a given situation, as is well documented in a number of human and animal studies where reliance upon a "dominant" modality in one situation may preclude the use of another quite sensitive system in that same circumstance. Further support for the notion of a highly olfactory *P. maniculatus* comes from studies demonstrating that females of this species are susceptible to olfactory-induced pregnancy blockage (Bruce Effect) (Bronson and Dezell, 1968; Bronson and Eleftheriou, 1963), as well as to male-induced synchrony of estrus (Whitten Effect) (Bronson & Marsden, 1964). To the author's knowledge, no accounts of similar phenomena occurring in *P. leucopus* have been published, to date.

SUMMARY

1. A review of the small rodent olfactory literature was undertaken and a hypothesis was formulated suggesting that some species of rodents may engage in courtship phenomena similar to that observed in many species of birds, fish and amphibia, with the salient stimuli involved being olfactory, rather than visual or auditory.

2. Studies 1 and 2 demonstrated that the stage of estrus can presumably influence odor preferences of female *P. maniculatus* mice for male-mouse odors of *P. maniculatus* and *P. leucopus*, when the odors are paired in a two-choice situation, and when they come from an accumulation of urine and feces, as well as from the male himself. However, the potential use of auditory cues in this type of test situation could not be entirely ruled out.

3. Study 3 demonstrated that cumulatively-aged urine from *P. maniculatus* males is attractive to *P. maniculatus* females, and may be somewhat attractive to *P. leucopus* ones as well, when the odor is paired with an air alternative. Female *P. maniculatus* showed a tendency to avoid the *P. leucopus* male urine. These effects were not markedly influenced by the estrous state of the females.

4. Study 4 found a strong position preference of the males for the right side of the olfactorium--a preference not found for the females of Study 3. A significant species x estrous state interaction was suggestive of a stronger attraction to estrous that to diestrous female urine odors by male _P. maniculatus_, and a stronger attraction to diestrous than to estrous female urine odors by male _P. leucopus_. However, separate analyses for each species did not disclose significant effects due to the estrous condition of the females producing the stimulus urine.

5. The number of crosses in the olfactorium during a test session by diestrous _P. leucopus_ females in the presence of _P. manicualtus_ male urine odor was found to be greater than the number of crosses by the females in the other test conditions (Study 3). In Studies 3 and 4, the number of crosses by both species was generally larger in the presence of heterospecific than in the presence of homospecific opposite-sexed odors.

6. No systematic relationship was discernable between the age of the cumulative stimulus urine build-up and the magnitude of the behavioral preferences or the number of crosses in the olfactorium. This was true for both male and female subjects (Studies 3 and 4).

7. Reliable modes of responding were observed across the test sessions of all studies, as evidenced by high intratest reliability coefficients for the percent time spent on a given side.

8. A comparison of the percent times spent with an opposite-sex urine odor by males and females suggested that female *P. maniculatus* reacted more strongly than male *P. maniculatus* to opposite-sex urine odors in directions that conceivably could result in a higher probability of encounters with conspecific opposite-sexed animals, and a lower probability of encounters with heterospecific ones. While *P. leucopus* estrous females were more attracted to opposite-sexed homospecific odors than were males of the same condition, similar sex differences were not found in the other testing conditions for this species. The sex difference found in this one group may be due primarily to a strong tendency of the male *P. leucopus* to avoid the odor of the female urine.

9. Evidence was presented suggesting that the contentions of the model, suggesting potential courtship phenomena in some rodents similar to that observed in some non-mammalian species, may be more applicable to *P. maniculatus* than to *P. leucopus*.

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