OLFACTORY PREFERENCES AND ETHOLOGICAL REPRODUCTIVE ISOLATION BETWEEN DEME POPULATION GROUPINGS OF FERAL HOUSE MICE (*MUS MUSCULUS*)

By

Thomas P. Cox

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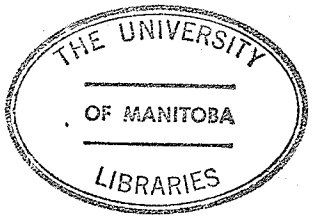

ABSTRACT

Some rodent populations are composed of smaller local populations or demes. Such demes are reproductively isolated even when they are in close proximity. Territoriality has been advanced as an isolating mechanism and olfactory variables are strongly implicated. This research investigated olfaction in the isolation of house mouse (Mus musculus) demes.

Experiment 1 provided male and female mice with a choice between fresh air and odors from males or females from the same or an adjacent deme. More time was spent with the same deme odors. The odors of members of an adjacent deme were avoided.

Experiment 2 tested reactions to urine and feces. These odors are assumed to be associated with territorial marking. Odors originated from males or females from the same or an adjacent deme. The total time and frequency of contact with an odor source were recorded. More time was spent with the odors of the same group. The odors of adjacent group males were contacted most frequently.

Experiment 3 was designed to determine whether the olfactory preferences displayed in Experiments 1 and 2 were reflected in the breeding choices of deme members. A male was confined with two females from his own deme and two from am adjacent deme (for each of three replications). The frequency of insemination, as evidenced by vaginal plugs, was the dependent measure. When the subjects were placed together aggressive encounters were common-

place. No breeding took place. Therefore, the frequency of aggressive encounters between members of the same deme as the male, or another adjacent deme was adopted as the dependent measure in lieu of the original measure. Results showed that aggression was twice as common between the members of two different demes as between the members of a single deme.

A second series of experiments (Experiments 4, 5 and 6) attempted to determine if the results of the first three experiments were a function of the physical proximity of the demes tested. Close proximity could increase the probability that the test animals had previously encountered either the adjacent deme members or their own odors. For this series of experiments, the "other" group stimulus odor animals were captured several miles distant from the trapping site which yielded the test subjects. Results for this series of experiments were very similar to those of the first experimental series. These results are discussed with relation to between deme sexual isolation, territoriality and genetic fitness.

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OLFACTORY PREFERENCES AND ETHOLOGICAL REPRODUCTIVE ISOLATION BETWEEN DEME POPULATION GROUPINGS OF FERAL HOUSE MICE (*MUS MUSCULUS*)

The production and detection of odorous substances are intimately involved in rodent reproduction. Both alterations to female reproductive physiology and the behavioral reactions to these changes have been well documented. A few of these reactions include sexual maturation, courtship, mating, post-copulatory events and parental behavior. Social status and spacing behaviors of individuals within a population are also related to reproductive activity, and olfactory variables are operative here as well.

The social structure of many small mammal populations is characterized by mechanisms which seem to limit the movement of individuals to a rigidly defined area. The behaviors associated with home range and territorial maintenance are generally considered responsible for this limitation to individual movement. It has also been recognized that breeding is restricted to individuals occupying territories. The territory then limits the number of potential matings which may occur within an area. Therefore, it must have a depressing effect on gene flow within a population.

Both behavioral and direct genetic evidence suggest that some rodent populations may be sub-divided into smaller inbreeding

groups or demes within the larger local population. It is possible that olfactory processes are involved in the establishment and/or maintenance of deme-type breeding units. The literature reviewed below will in general consider olfactory involvements in rodent reproductive behavior. The question of the involvement of these stimuli in spacing and inter-and intraspecific sexual isolation will receive particular attention. A series of experiments designed to assess the role olfactory preferences play in intraspecific sexual isolation will then be described.

Olfactory Involvements in Reproductive Behavior

Sexual Maturation

A close relationship between olfaction, sexual behavior and endocrine functioning has been demonstrated for a number of rodent species. One such relationship is that between olfactory stimuli and the reproductive physiology of the female house mouse (*Mus musculus*). For instance, Vandenberg (1969) has reported that the sexual maturation of females is hastened by exposure to the odor of adult male mice. The age at which a female mouse first displays estrous is inversely related to the duration of exposure.

Acceleration of female maturation was achieved when females were exposed to the soiled bedding of male mice. This indicates that the effect is odor-induced. The effect was most pronounced when the females were exposed to the bedding of male mice which had been housed near them, either in the same cage or separated

by a wire mesh partition. This effect could also be produced by exposing immature females to the odor of adult female mice. However, in this second case the effect was not significant. Unfortunately, Vandenberg did not expose his females to control odors obtained from males housed near diestrous females or other males. Therefore, it is difficult to determine the specific factors of the stimulus odor which may contribute to this process.

Cycling of Receptivity

Grouping of females. The close relationship between olfaction and reproductive physiology has been further demonstrated by a number of studies which link olfactory variables to periods of female sexual receptivity. Typically, estrous cycling (heat) is a regularly recurring period of female sexual receptivity which occurs each four to five days. The intervening time between periods of estrous is one of low sexual receptivity known as diestrous or anestrous.

Typical estrous periodicity can be altered by prolonging the entire cycle. This lengthening of the estrous-to- estrous period can occur by a) prolonging the diestrous portion of the cycle, or b) inducement of pseudo-pregnancy. Whitten (1955) has been able to demonstrate both of these effects. He showed that pseudo-pregnancy could be induced when female mice were housed in small groups (N = 4). Whitten (1956a, 1956b) also found that estrous cycling was eliminated by an olfactory bulbectomy. This operation also resulted in a reduction of ovary size. Other researchers (Lee & Boot, 1956) later demonstrated that odor mediated the

prolonging of estrous cycling in groups of female mice. They reported that this effect could not be reproduced in bulbectomized subjects. However, this operation does not markedly influence the estrous cycling of rats (Rosen, Shelesyak & Zacharias, 1940) or guinea pigs (Donovan & Koprina, 1965).

Whitten (1959) has also reported that the grouping of female mice (group N = 30) can suppress female estrous by producing a period of prolonged diestrous. Parks and Bruce (1961) found that physical contact was not necessary to to produce this effect.

Male-induced estrous synchrony. The influence of a male mouse's odor upon the female's reproductive physiology is perhaps the best known interaction between olfactory variables and endocrine processes. Numerous authors (Bronson & Marsden, 1964; Chipman & Fox, 1966; Marsden & Bronson, 1964; Whitten, 1956a; Whitten, et al., 1968) have reported that the odor of a male mouse can shorten (accelerate) the intervening time between periods of female sexual receptivity. When groups of females are exposed to the odor of a male the acceleration of their estrous cycles can result in an increased number of females coming into heat on the third night night following the odor presentation. This is called a synchronization of estrous cycling. Just the presence of a male mouse in an animal room can provide sufficient stimulation to influence the estrous cycling of female mice. This effect has been termed the "Whitten effect."

It is generally believed that this synchronization of estrous cycling may be mediated by the air-transported odors of male bladder urine (Brown-Grant, 1966; Whitten, et al., 1968). Bron-

son and Whitten (1968) report that the source of the estrous synchronizing odor is not preputial in origin, but Gaunt (1968) was able to reproduce the effect by administering preputial homogenates.

Scott and Pfaff (1969) found that castration eliminates the compound from the male's urine which produces estrous-synchrony. Testosterone replacement treatments evidently restore this compound, and consequently its estrous-synchronization effect, to the male's urine. Scott and Pfaff also reported that female mice spend more time sniffing the odor of normal male urine than that produced by castrated males. Findings of this sort prompted Bronson and Whitten (1968) to speculate that the urine compound which produces estrous-synchronization is either a product of androgen- maintained tissue or an androgen metabolite.

Discrimination of, and Preference for Different Phases of Estrous

Not only can olfactory stimuli alter reproductive physiology, but hormonal states may influence sexual preference. For instance, Carr and Caul (1962) found that sexually-experienced male rats prefer the odor of an estrous female to that of a diestrous female rat. Several authors (Carr & Caul, 1962; Carr & Pender, 1958; Carr, Solberg & Pfaffman, 1962) have also reported that castration eliminated this preference. However, the castration does not eliminate the male rat's ability to discriminate the estrous state.

The available data suggest that sexual experience is required before a male rat will show a preference for the odor of estrous

conspecifics (Carr, Loeb & Dissinger, 1965; Stern, 1970). The data presented by Pfaff and Pfaffman (1969) support this position, but their results also indicate that the odor of female urine alone may be sufficient to produce this preference. However, these authors used an experimental design which confounded the sexual experience and castration variables. This makes their conclusions difficult to interpret.

The preference of female for male rats may also be dependent upon reproductive physiology. Diestrous, sexually naive females did not display a preference for the odor of either normal or castrated males, but both sexually experienced and estrous females consistently preferred the odor of normal males (Carr et al., 1965).

Carr, Wylie and Loeb (1970) have investigated the homotypical (same sex) odor preferences of rats. Male rats showed no preference for the odor of other male rats over all combinations of sexual-experience and gonadal states. However, sexually-naive estrous females preferred the odor of non-receptive females. This odor preference was not maintained for either sexually experienced or naive diestrous females.

Copulatory Behavior

Some evidence indicates that olfactory processes may mediate the consummatory portions of rodent sexual behavior (copulation) as well as their reproductive physiology. This was demonstrated by the elimination of mounting behavior in both male (Murphy & Schneider, 1970) and early-androgenized female (Doty, Carter & Clemens, 1971) hamsters whose olfactory bulbs had been removed.

Rats which had received this operation showed a reduction in their ejaculation frequency (Beach, 1942; Heimer & Larssor, 1967). However, previous copulatory experience may be an important factor for this species, for postoperatively the males which had prior sexual experience displayed increased times to ejaculation, and their inter-intromission latencies did not differ from those of normal rats. Inexperienced male rats were deficient in both measures (Bermant & Taylor, 1969).

Post-Copulatory Physiological Effects

Several authors (Bowers & Alexander, 1967; Husted & McKenna, 1966) have described the ability of rats and mice to use olfactory cues to discriminate between individuals. This topic will be discussed later. However, this process of inter-individual identification may mediate a phenomena known as the "Bruce effect." It was Bruce who first described a process in which the odor of a strange (non-stud) male could prevent ova inplantation in recently inseminated female mice (Mus) (Bruce, 1959, 1960, 1965). Following a pregnancy blockage the female mice returned to their normal estrous cycling. The effect has also been described for deer mice, Peromyscus (Bronson, Eleftheriou & Garrick, 1964) and meadow mice, Microtus pennsylvanicus (Mallory, Clulow & Langford, 1971).

When bulbectomized females did not show the "Bruce effect," Bruce and Parrott (1960) intimated that olfactory processes might mediate this behavior. Their supposition was supported when it was found that the effect could be duplicated by exposing inseminated females to the urine of previously unfamiliar males

(Bruce, 1965; Bruce & Parrott, 1960; Dominic, 1964, 1965, 1966). An individual recognition process is probably a factor contributing to this effect, for re-exposure to the original male failed to block (for a second time) the pregnancy. Since the odors of females or castrated males do not produce a pregnancy block this process could be dependent upon the gonadal condition of the male.

The Question of Infraspecific Population Units

Reproductive Isolating Mechanisms

A major concern of evolutionary theory has been the identification of those factors which maintain a species' identity, in other words, the process of species formation. This topic involves a phenomenon known as reproductive isolation. The term reproductive isolation refers to the means by which genetic interchange between potentially inbreeding populations is prevented or hindered. There are several ways in which reproductive isolation can be achieved. For example, behavioral or physiological mechanisms may effectively prevent copulation between the members of two species or, if mating does occur, other mechanisms can come into play which will prevent the production of offspring. Also, if young are produced, the hybrid decendents of the mating could themselves be less viable or less fertile than the products of a within-group mating. The following discussion will be concerned with the first of these reproductive isolating mechanisms, the pre-mating isolation mechanisms.

Pre-mating sexual isolation can be achieved in three ways.

First, the members of potentially interbreeding populations can have differing habitat preferences or requirements. Therefore, they may only occasionally meet one another and there would only be a few occasions when interbreeding could occur. Also, potential mates could become sexually receptive at different times of the year. If this happened the populations would be considered to be seasonally isolated. Another possibility, and the one most intimately connected with the theme of the following review, is that the hypothetical species or populations may possess certain behavioral mechanisms which dictate a preference for intraspecific or intra-population matings. This last form of reproductive isolation is often referred to as "ethological" or "behavioral" isolation.

There are, of course, a number of ways in which ethological isolation could be achieved. In light of the literature reviewed above it could be assumed that one of these is a species specific differential sensitivity to chemical stimuli. In other words, the members of a species may be attracted only by the odor of homospecifics, while the odors of heterospecifics are neutral or aversive. Ehrman (1969) and Ewing and Manning (1967) have presented evidence that such a process may operate for Drosophila. Similar olfactory-mediated sexual isolation has also been described for Sceloporus lizards (Hunsaker, 1962) and some rodent species (Doty, 1972, 1973; Godfrey, 1958; Moore, 1965; Smith, 1965).

Infraspecific Reproductive Isolation

The study of reproductive isolation has usually been limited

to interactions between species. However, since evolution must also occur at infraspecific levels, it is reasonable to assume that similar mechanisms regulate breeding within the species. The research discussed below is concerned with the existence of olfactory- mediated sexual isolation between infraspecific population units. The first topic to be reviewed in introducing this research will be the question of the existence of these infraspecific population units.

Wright (1931) in an early theoretical paper discussed several hypothetical genetic structures of populations below the species level. The first of these was termed the "isolation by distance" model. It assumed that populations were not divided into subunits. In this model the physical limits of the dispersal distances of individuals within a local population were the only determinants of breeding patterns for the population as a whole. In the second, or "island" model, populations were divided into distinct inbreeding units called demes. Within a deme mating could be random, but physical barriers or behavioral segregating mechanisms tended to isolate a deme within the greater population. Thus, behavioral isolating mechanisms could limit between-deme reproduction, perpetuating and augmenting the isolation effect. An investigation of some of these behavioral isolating mechanisms which perpetuate a deme type population division is a major theme of this research.

Individual Movement

Patterns of dispersal. The members of many small mammal populations can be considered as either dispersing or non-

dispersing individuals. After weaning, the young of many small mouse-like mammals become transient, dispersing from the area of their birth (Calhoun & Webb, 1953; Getz, 1961; Terman, 1961). This dispersal has been explained in a variety of ways. For instance, Krebs, Gains, Keller, Myers and Tamarin (1973) reiterated a hypothesis presented earlier by Chitty (1967) who contended that some members of a population possessed a genetic predisposition for dispersal. On the other hand, Christian and Davies (1964) have presented evidence which suggests that certain behavioral-physiological mechanisms may be sensitive to population growth. The mechanisms of dispersal are not however, a concern of this paper.

Area restricted movement. The nondispersing members of the population evidently spend a considerable, but unknown, proportion of their life within a relatively restricted area (Beer, 1961; Getz, 1961; Watts, 1970). This area is usually referred to as a home range. Although there is some controversy surrounding the proper definition of the term, it generally refers to the area used by the animal as it goes about its normal day-to-day activities (Burt, 1943; Dice, 1952). These home ranges, of course, vary in size depending upon the species, the habitat and the population they support. Some typical home range estimates for some of the more common rodents are as follows: meadow vole, Microtus pennsylvanicus, 0.02-0.58 acres (Brown, 1975); deer mouse, Peromyscus maniculatus, 0.74-1.67 acres (Brown, 1975) and the feral house mouse, Mus musculus, 0.0029-0.33 acres (Quadango, 1968).

Aggression as an Infraspecific Isolating Mechanism

Not only do mice live in a restricted area, but when predation is low and the food supply adequate, they will remain there for fairly long periods of time. Anderson (1964, 1967) has reported that in such an environment house mice, Mus musculus, can remain within their restricted home ranges for at least a year. Reimer and Petras (1967) have produced similar results. If these relatively permanent home ranges are used fairly exclusively by a mouse or group of mice, and if other mice are not allowed into the area (that is, the home ranges are territories), then it might be expected that the limited movement between areas would have a depressing effect on gene flow within the larger population. A number of studies have demonstrated that strange mice are not readily integrated into established populations (Anderson, 1964, 1967; Anderson & Hill, 1965; Brown, 1953; Calhoun & Webb, 1953; Crowcroft & Rowe, 1963; Murray, 1967; Reimer & Petras, 1967; Wolfe & Summerlin, 1968). Several of these authors, and others, have implicated a territorial system maintained by interpersonal aggression in this segregation effect (Crowcroft, 1955; Crowcroft & Rowe, 1963; Davis, 1958; Wolfe & Summerlin, 1968). In addition, several authors (Anderson, 1964, 1967; Anderson & Hill, 1965; Rasmussen, 1964; Reimer & Petras, 1967, 1968; Sealander & Yang, 1969; Sealander, 1970) have suggested that this antagonism towards strangers and territorial maintenance may isolate a population into smaller breeding units. The above evidence indicates that Wright's "island" model may

best illustrate the mating structure of some rodent populations. Such inbreeding infraspecific populations are commonly referred to as demes.

The house mouse (Mus musculus) is often cited as an example of a species which exhibits a deme-type breeding pattern conforming to Wright's "island" model (Anderson, 1964; Sealander, 1970). Territorial behavior is generally considered to be the behavioral mechanism which isolates the individual population units. As has been mentioned above, house mice do display area-related dominance. In fact, Eibl-Eibesfeldt (1950) has described the territories of house mice as communal, that is they are defended by an extended family or tribe. Behaviors of this type could probably isolate a larger population into inbreeding subunits even in the absence of physical barriers to inter-deme migration.

Reimer and Petras (1967) have tested the notion that behavioral limiters to individual movement such as territorial defence can isolate potentially interbreeding populations of house mice, Mus musculus, into deme-like units. In this study the mice were housed in a "population cage" which consisted of a series of 16 nest boxes connected by a runway complex. The subjects, mice of genetically distinct origins, were introduced into the individual nest boxes in genetically mixed groups. All the subjects and their eventual offspring were marked and their location checked daily. In a short while the mice began to form groups or "tribes". These "tribes" were made up of a dominant male and the animals of either sex which allied with him in the defense of a nest box, or series of nest boxes. The groups were not allied

according to their genetic origins, and contrary to the reports of Anderson and Hill, the females aided in defense of the tribal nesting area.

Reimer and Petras found that once the boundaries of a tribal territory were established, they remained remarkably stable over a number of generations. The boundary stability remained even as the population increased tenfold to a density of 10 mice/square foot. A problem with this study is that although groups of animals remained in defined areas for long periods of time, Reimer and Petras inferred that only territorial defense behaviors maintained the segregation. Their inferences were based upon occasional observations of aggressive encounters, number of wounds, and absence of movement between areas. Circumstantial as it is, Reimer and Petras concluded from these data that the animals found within each defended area constituted a stable inbreeding family unit or deme.

As has been mentioned, Reimer and Petras speculated that the segregation between groups of mice in their study could have been induced by the territorial exclusion of non-group members. This type of social behavior has been discussed as important in initiating isolation between small local gene pools (Anderson & Hill, 1965; Christian, 1970; Reimer & Petras, 1967). Thiessen and Dawber (1972) have demonstrated that this may indeed be the case. In their experiments territorial Mongolian gerbils (Meriones unguiculatus) excluded strangers from their "home territory". The intruders were forced to cross a water barrier to safety even though gerbils rarely enter the water when allowed

a choice. Thiessen and Dawber also found that animals which had emigrated subsequently avoid the odor of the animal from whose territory they had been excluded. The above inferences have been lent further support from the electrophoretic analysis of the breeding patterns of feral house mouse populations.

Electrophoretic Evidence

The genetic structure of the house mouse, Mus musculus, has been studied more than that of any other species of small mammal. Gel electrophoretic procedures allow the detection of fine-scale variation at a number of gene loci which control the structure of hemoglobin and certain enzymatic proteins. The relative frequency of specific electrophoretically detected genetic markers within a population is generally considered to be indicative of the inter-intra group breeding patterns of that population (Rasmussen, 1964).

Recent studies of house mice populations have shown genetic heterogeneity between populations living in adjacent barns (Anderson, 1964, 1967; Lewontin & Dunn, 1960; Petras, 1967a, 1967b; Reimer & Petras, 1968; Sealander, 1970), and even within the same barn (Sealander, 1970). Although house mice do display clinal genetic differentiation, this factor can probably be rejected as an explanation of the stable between-population electrophoretic variation described because these differences have been detected for mice living within the same building.

All the above evidence indirectly supports a hypothesis of Peromyscus local breeding structures advanced by Blair in 1953. Trap records of the dispersal patterns and of individual mouse

movements within a population occupying a continuous habitat suggested to Blair that the breeding structure of the population was composed of inbreeding family units of relatively small size. Several studies (Anderson, 1964, 1967; Reimer & Petras, 1968; Sealander, 1970) have predicted that a deme may be composed of as few as ten individuals. As was indicated above, these authors have also suggested that territorial and home range maintenance behaviors may be responsible for the long-term division of a population into demetic units.

If interpersonal aggression and territorial defense behaviors are responsible for mating segregation between infraspecific population units, then it may be asked; "How are strangers or their territories recognized?." In other words, what stimulus components are responsible for the maintenance of a deme-like breeding system?

Olfaction as an Intrademe Isolating Mechanism

Several authors have reported that sniffing increases in frequency prior to an aggressive confrontation between mice (Banks, 1962; Lloyd & Christain, 1967). The aggressive defense of a territory has generally been attributed to the males of a population (Reimer & Petras, 1967; MacKintosh, 1970). Archer (1968) has shown that an aggressive reaction is most likely when a male mouse is exposed to the odor of a strange male. Also, if a mouse is taken from a previously established hierarchy, rubbed with the urine of a strange male and returned to the group, it will be met with aggression by other members of the group. In addition, there is ample evidence showing that dominant males

urine-mark an area more frequently than do subordinate mice (Desjardins, Maruniak & Bronson, 1973; Eisenberg, 1962, 1963; Ralls, 1971). Marking may take the form of depositing sebaceous gland secretion upon objects in the environment as the Mongolian gerbil does. Other rodents like the deer mouse, Peromyscus maniculatus, mark with urine. Eisenberg (1962) reported that some species of Peromyscus use this means of marking all areas of potential contact with strangers. He (Eisenberg, 1963) believed that in the wild marked areas "must be considered as areas of information exchange; several animals living in proximity could communicate their individuality and reproductive condition without ever coming into physical contact (p. 41)."

These "marked areas" are often considered indicative of territorial boundaries. Mice are reported to be reluctant to cross established territorial boundaries (Reimer & Petras, 1967; MacKintosh, 1970). Nyby, Thiessen and Wallace (1970) found that for gerbils, Meriones unguiculatus, the reduction in the marking behavior of subordinates was olfactorily mediated. It has been suggested that the secretions of the preputial gland are the source of this odor (McKinny & Christain, 1970; Mugford & Nowell, 1970). These secretions have also been reported as highly attractive to female conspecifics (Bronson & Caroom, 1971).

As has been noted, many aspects of rodent reproduction seem to be mediated by olfactory cues. The literature reviewed above has linked olfactory stimuli to the territorial defense related behaviors of boundary marking, interpersonal aggression and dominance. These behaviors have in turn been discussed in

relation to within-population mating patterns. An important next question might be; "What processes maintain reproductive isolation between these population subunits?." One obvious possibility is the olfactory identification and separation of subspecific group members. Before the olfactory isolation of demes is considered, it might be instructive to review evidence of the inter- and intraspecific discriminations and preferences of rodents.

Reproductive Behavior

Interspecific Effects

Interspecific discrimination. It was mentioned earlier that there is evidence which indicates that rodents can recognize members of their own species on the basis of olfactory cues. This ability was first demonstrated by Bowers and Alexander (1967). They presented adult mice (Mus musculus) with a simple Y-maze discrimination task. The adult mice of either sex were able to choose between the odors of male house mice (Mus musculus) and deer mice (Peromyscus maniculatus) when reinforced with drinking water. These findings were partially confirmed by Hahn and Simmel (1968). However, their study did not report the sex of the stimulus mouse. Also, the presence of visual and auditory cues make an exclusively olfactory interpretation impossible. This study did, however, suggest that the long term accumulation of urine and fecal matter, as used by Bowers and Alexander, is not a prerequisite for forming the discrimination.

Although mice can discriminate species membership using only

olfactory cues, this discriminatory ability does not necessarily mean that olfactory cues actually influence mate selection, and thus operate as an isolating mechanism. A look at rodent preferences might partially clear up this problem.

Interspecific preferences. Several studies have suggested that olfactory cues may be important in influencing conspecific mate selection. For instance, Smith (1965) examined the time spent by allopatric and sympatric male cactus mice, Peromyscus eremicus, and California mice, Peromyscus californicus, in three middle compartments of a linearly arranged five compartment chamber. The extreme compartments of Smith's test chamber were separated from the adjacent compartments by wire mesh. A homospecific female was housed in one of these end chambers and a heterospecific female in the other. The sympatric male cactus mice, Peromyscus ereicus, spent most time near the homospecific female, an intermediate amount of time in the neutral compartment and the least time in the compartment nearest the heterospecific female. The allopatric males displayed an opposite reaction. They spent less time near the homospecific females and more time near the heterospecific ones than did the sympatric males. These data tend to support McCarley's (1964) suggestion that in some species isolating mechanisms are reinforced in sympatric areas. One of the flaws of Smith's study was that he did not report the estrous condition of the stimulus females. These findings would perhaps have been of more value had the reactions to both estrous and diestrous females been examined.

In a similar study, Moore (1965) found that both sexes of the

deer mouse, Peromyscus maniculatus rufinus, spent more time in the area of a three-compartment chamber which had previously housed a homospecific mouse for eight hours. This area was preferred over one which had been occupied by a heterospecific old field mouse, Peromyscus policnotus, for a similar period. Because Moore's study used previous occupancy as a stimulus rather than another animal, his results are more compatable with an olfactory hypothesis than are Smith's. Also, Moore's results are more interpretable than are Smith's because Moore used only estrous females in his study.

The studies reviewed above have given more attention to the olfactory preferences of the male rodent than to those of the female rodent. A statement by Godfrey (1958) perhaps explains this emphasis. He wrote, "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)." Reacting to the neglect of the female rodent within the olfactory literature, Doty (1972, 1973) designed a series of studies to investgate the olfactory preferences of sympatric species of Peromyscus females.

Doty's (1972, 1973) studies found that estrous female deer mice, Peromyscus maniculatus, preferred the odor of homospecifics. However, female white-footed mice, Peromycus leucopus, failed to show a differential reaction to the odors of either species. Doty suggested that only some species, in this case Peromyscus maniculatus, utilize urine-transported odors as an isolating mechanism. Doty also found that male white-footed mice

avoided the odcr of males of both species. These data perhaps relate to the aggression and territorial marking literature mentioned earlier.

With the exception of Doty's somewhat confusing results, it does appear that at least some species of rodents within the genus Peromyscus are able to utilize odor for intra- and interspecific communication. In fact, the preferences reported by both Moore and Smith indicate that olfaction could be the basis of interspecific sexual isolation for at least one group of rodents.

Intraspecific Effects

Subspecific preferences. In a previous section it was mentioned that olfactory stimuli may contribute to reproductive isolation below the species level. Godfrey (1958) presented evidence which indicated that bank voles, Clethrionomys, may be reproductively isolated at a subspecific level. He presented the males of two geographically distant forms (races) of Clethrionomys britannicus (Kintyre and Edinburgh races) with a group of females, half of which were examined for indications of mating activity (vaginal plugs). It was found that the males of both races selectively inseminated the females of their own race.

Godfrey suggested that these differential rates of insemination might be mediated by olfactory processes. To test this notion he presented the odors of two groups (presumably different species, or races on the verge of speciation) of female bank voles to male voles in a Y-maze situation. The males approached the odor of homospecific females in preference to the alternate

odor. Also, in some instances, the males chose the odor of the females from their own locality in preference to females from other localities. Tests which used hybrid voles for stimulus odors indicated that these animals were usually discriminated against.

Although Godfrey's data for intra-racial olfactory discrimination were not conclusive, they do suggest that olfactory processes may be involved in subspecific reproductive isolation. A problem with this supposition is that the Clethrionomys used by Godfrey originated on widely separated islands and were probably on the verge of speciation. In fact, several taxonomists have treated the local races used by Godfrey as full species (Godfrey, 1958). Therefore, there is some doubt that this study is really an example of subspecific ethological reproductive isolation.

Many of the studies reviewed above have indicated that some rodents are capable of making fine olfactory discriminations, However, only Godfrey has presented evidence for subspecific olfactory discriminations. Although no study has actually dealt specifically with the olfactory segregation of population groupings, there is evidence which indicates that some infraspecific differences can be detected by rodents.

Interindividual discrimination. The olfactory discrimination of the members of an infraspecific population unit could be based upon one of, or a combination of two processes. Recognition could be due either to genetically determined differences in odor production or detection, or to the identification of separate olfactory identities for individuals within a population. Most

of the interspecific discrimination and preference data which have been reviewed so far favor the first of these possibilities. However, several studies have indicated that rodents may alsc be able to detect individual differences in odor. For instance, Husted and McKenna (1966) demonstrated that in an operant situation rats could learn a discriminatory response in the presence of a second rat. Although these authors did not specifically test for the operation of olfactory variables, they did report that the rats directed a great deal of sniffing behavior toward the stimulus animal's anal region. Husted and McKenna further suggested that olfactory stimuli were important cues for the forming of the discrimination.

Bowers and Alexander (1967) have reported the results of an experiment which dealt more directly with the possibility that olfactory processes may mediate interindividual discriminaticns. In this study C57Bl mice, Mus musculus, were rewarded with drinking water when the "correct" choice between twc nonrelated mice of the same inbred strain was made. These authors reported that all animals tested were able to perform the discrimination. In a similar study, Hahn and Simmel (1968) replicated the results reported by Bowers and Alexander (1967). However, Hahn and Simmel also found that male mice, Mus musculus, discriminated between individual litter mates. Unfortunately, this study did not exclude possible visual and auditory cues. This omission makes a strictly olfactory interpretation impossible.

It is possible that individual recognition processes may mediate a number of the olfactory-linked phenomena which have

been described. For instance, certain physiological processes like the Bruce effect could be triggered by the recognition or nonrecognition of certain individual odor complexes. Also, as has been discussed, dominance posession of an area, group membership and aggression seem to have an olfactory component. Rodents may well depend upon the relative olfactory "familiarity" of an individual to monitor the social structure of their population.

As has been pointed out, many aspects of rodent behavior are mediated by the olfactory detection of chemical stimuli. All of these processes, including the social aspects of rodent behavior, are intimately related to rodent reproduction, and therefore to the evolutionary history of the species. Previous discussion has indicated that many aspects of rodent reproduction such as the avoidance of, or attraction to, potential mates may consequently influence the breeding patterns of the species. These processes may in turn be determined by patterns of individual recognition of local population membership. Therefore, it appears as though olfactory processes may mediate, or enhance, the relative sexual isolation between local sub-units of a larger population. This is the possibility to which the following research is directed.

Purpose

The literature reviewed in the preceding section suggested that limits to individual movement imposed by territorial and home range maintenance behaviors may isolate some small mammals into inbreeding subunits within the larger general population.

Electrophoretic analysis techniques have revealed that genetically distinct infraspecific breeding units do exist for some species of wild mice. Indeed, several authors contend that these smaller breeding units are maintained by the movement limiters mentioned above (Anderson, 1964; Anderson & Hill, 1965; Rasmussen, 1964; Reimer & Petras, 1967, 1968; Sealander & Yang, 1969; Sealander, 1970).

Many of the studies reviewed above have attempted to demonstrate segregation between groups of rodents on the basis of odor production or detection. However, these olfactory studies are somewhat limited in their attempts to identify the ecological-behavioral factors which influence the process of speciation to which the question of infraspecific reproductive isolation is inextricably linked. One factor which has limited the generality of these studies' findings is their general use of inbred strains. This becomes a special limitation in light of evidence which suggests that the olfactory identity of the house mouse (Mus musculus) may be reduced or eliminated by inbreeding (Bruce, 1968). Also, the Smith (1965) study which presumed to assess olfactory processes as a possible interspecific isolating mechanism did not clearly exclude other potential identification cues such as auditory and visual stimuli. Finally, the majority of studies which have attempted to implicate olfactory variables in the speciation process and which made use of wild populations have only considered the possibility of olfactory isolating mechanisms as operating between genus and/or species (Bowers & Alexander, 1967; Doty, 1972, 1973,; Moore, 1965; Smith, 1965).

As Wilcock (1972, p. 533) has commented, "Since it is within species that evolutionary process is taking place, it follows that some understanding of behavioral evolution must arise through comparison within species." Earlier olfaction studies did not really address this aspect of the problem. That is, to examine adequately stimuli or processes which may operate as sexual isolating mechanisms in species formation, it is necessary to focus attention upon those aspects of a population within which ethological isolation would be most meaningful. If evolution is a dynamic process, behaviors maintaining the distinction beween species must be manifest below the species level.

It is possible that olfaction is involved in infraspecific sexual isolation. Several studies have implicated olfactory processes in various social interactions among rodent conspecifics. These social interactions include the very movement-limiting devices mentioned earlier. Olfactory variables have been implicated in rodent aggression (Archer, 1968; Lee, 1970; Mugford & Nowell, 1970) and dominance (Carr, Martorano, & Krames, 1970; Desjardins, Maruniak & Bronson, 1973). Such olfactory-mediated component behaviors may be the basis for, or at least reinforce, the territorial maintenance which is believed involved in the formation and segregation of infraspecific population units (Nyby et al., 1970; Thiessen & Dawber, 1972).

The only study which approaches an adequate assessment of the relationship between olfactory preferences and mating choice at subspecific levels is Godfrey's (1958). Godfrey's inclusion of differential insemination data certainly allowed a closer look at

the possible relationship between olfactory variables and the actual breeding patterns contributing to the species forming process. However, as with other studies in this area, Godfrey may have been evaluating potential isolation between groups which were already quite distinct. Godfrey himself noted that many taxonomists have classified the "subspecific" forms he used in his tests as "full species." And Godfrey classified these forms as being at least on the "verge of speciation."

To summarize, certain rodent species seem to possess a well-defined potential for fine olfactory discriminations. Also, there is a relationship between olfactory variables and certain rodent social behaviors. Finally, these behaviors seem to be related to the kinds of mechanisms suggested as important in intraspecific sexual isolation processes. If these behavioral relationships are connected in this way, it then becomes important to reexamine this area in an attempt to define the real relationships between these variables. Therefore, the purposes of this series of studies were as follows:

(a) to determine if the members of a single rodent species can discriminate population membership using only olfactory cues;

(b) to determine if this phenomenon occurs in wild populations;

(c) to determine if these olfactory preferences are reflected in actual mating patterns;

(d) to determine if olfactory components in territorial and/or aggressive behaviors could function to maintain

different population preferences.

EXPERIMENT 1

A complex of behaviors may segregate a population into smaller deme-like breeding units. Several considerations are involved in determining if rodents show a differential preference between members of geographically distinct populations when only odor cues are available. First, conventional methods of determining an individual's preference between, for example, homo- and heterospecific groups generally involve a subject choosing one of several stimulus odors. Unfortunately, an experimental design which presents odors simultaneously possesses some built-in limitations. The response to one odor may be influenced by the presence of the other odor(s). For instance, it is conceivable that the odor of an estrous female when paired with that of a male could alter the expected reaction to either, or both odors. Also, it was possible that the physical characteristics of one test odor could alter the other odor or mix with it in some manner. The possibility of alterations to the subjects' sensory processes by exposure to an odor complex or mixture was another possibility that couldnot be dismissed.

A second problem with this type of choice methodology was that "preference" was defined by subject's spending more time with one odor source than another. This does not allow one to say whether this reflects a preference for that odor and/or an aversion to the other. One way of minimizing these potential problems was to pair each stimulus odor with air, rather than another animal's

odor. In this way attraction and avoidance could easily be scored. Also, the problems of odor mixing or alteration were reduced to the combination of a stimulus odor and presumably neutral fresh air. An added benefit of this design was that a situation involving a choice between two individuals was probably an unlikely event in the wild. A more common choice would probably be one of approaching or avoiding another individual or area.

Since this study is concerned with interpopulation reactions within the confines of the theories discussed earlier, test population sampling could represent an important problem. The samplings must be as nearly identical as possible and yet represent distinct, potentially inbreeding units. The data discussed previously suggest that the typical rodent behaviors of home range and territorial maintenance offer a convenient solution to this problem. If test animals were trapped from geographically distinct areas, (for instance between adjacent farm buildings as in the Anderson, 1964; and the Reimer & Petras, 1968 studies), completely foreign groupings could be obtained. To assure that the populations tested were indeed distinct social groups, a trapping, marking, and retrapping program was carried out.

The primary purposes of Experiment 1 were as follows:

(a) to determine if differential preferences exist between populations as a function of the odors produced by population members.

(b) to examine patterns of relative attraction and aversion

accompanying these preferences.

Method

Subjects

Because this series of studies deals with a possible movement-limiting mechanism between autonomous, naturally occuring groups of house mice, all subjects were live trapped. Several live trapping procedures are commonly used for small mammal population studies. However, none of these is especially applicable for describing the spatial relationships between individuals and the groups they compose. Methods designed for small mammal population studies, including that suggested by the International Biological Program (IBP), are such that the quadrants themselves as well as the recommended trap-to-trap distances are too great for accurate monitoring of relatively small-scale movements within the general population. In fact, an assumed qualification imposed upon these trapping methods is that the area trapped must be larger than the home range of the animals being studied. This limitation is especially apparent when the populations under study are as small as the estimated size of the sub-population groups this study is concerned with. Petras (1967a, 1967b) has estimated the size of a deme to range between 5 and 80 individuals. The findings of Anderson (1965) and Sealander (1970) agree with these estimates. In addition, Reimer and Petras (1968) have reported the distance moved by marked deme members varies between trappings. If these distances are related to the area occupied by a deme it is clear that a relatively

small area must be trapped if the objective involves collecting only the members of a single deme in the most practical manner.

A review of trapping methods employed in small group investigations indicates that both grid distance and the trap retrap periods are pragmatically determined by the individual investigators (for example, see Calhoun and Webb, 1953; Reimer and Petras, 1968; Rasmussen, 1964; Sealander, 1970). The trapping method used in the series of studies reported here was designed as a practical solution to the particular sampling required by this study.

As indicated, conventional population sampling methods are not well suited to deal with relatively small, sub-population groupings.. Thus, one should consider some possible requisites for trapping programs designed to investigate those smaller scale spatial relationships. First, a trapping program which attempts to capture, or monitor small groups within the general population, such as the hypothetical tribes or demes this research is concerned with, must encompass an area large enough to cover most of the area utilized by the group's members. In addition, the trapping area must be small enough to fall well within the estimated home range of the groups being considered. Also, because of the area's population density, a relatively high trap density, (ie. low trap-to-trap distance) must be used so that most of the groups' members can be captured.

Time limits for the trap-retrap periods also have a theoretical limit. These periods must be long enough to allow the majority of transients to pass through the area without encount-

ering both the trap and retrap periods. Also, some mechanism must be devised to minimize the possibility of including the transient members of neighboring demes in the sub-population sample. The probability of capturing and recapturing these neighboring deme members would increase with longer trapping periods. However, Anderson (1965) and Sealander (1970) have reported deme-type population groupings so stable that this final limitation may not actually present a problem.

The trapping procedure used in this study was designed with these particular qualifications in mind. Live trapping was conducted both within and between granaries. Traps set within granaries were placed at approximately one meter intervals about the inside perimeter of the building, and in any other location which appeared promising (on overhead rafters, etc.). The same general placement scheme was used for locating traps outside the building. In addition, four traps (two rows of two traps each) were placed between the granaries. Live traps were of two types; a metal trap of the "Sherman" variey and home-made masonite traps. Both traps measured approximately 23 cm. x 7.5 cm. x 7.5 cm. Traps were alternated in placement according to the type of trap constuction. This procedure eliminated the trapping grid problems discussed earlier.

House mice trapped within each granary were marked by toe clipping and released. The single trap location of each marked mouse was recorded for each subsequent recapture. Using this procedure, and following each mouse for 16 or more recaptures (and thirty or more days at each location), the home range of

each individual mouse could be defined. This procedure minimized the possibility of counting transients as members of the study population. In all cases house mice were captured only within granaries. Individual mice apparently used the entire building as a home range. However, in no case were mice found crossing from one granary to another. This is remarkable considering the relatively small distance separating the granaries (two to eight meters) at a trapping location. This finding was consistent with those of Anderson (1965) and Sealander (1970) mentioned earlier.

As mentioned above, the subjects themselves were acquired by live trapping. There were three sets of subjects. These were reproductively mature male and female house mice (Mus musculus). Each set was live trapped in separate locations in southern Manitoba. Two trapping sites were located in the Winnipeg region and are designated as: Township 9, Range 3 East, Section 19 and Section 30. These trapping sites were separated by about 1.5 miles. The third trapping location was located about 30 miles south of the first two and is described as: Township 7, Range 2 East, Section 13.

Each subject set of populations was composed of animals trapped in two (and at the third location, three) adjacent wooden farm granaries separated from one ancther by two to eight meters. The mice inhabiting a single granary at a trapping site are collectively or individually hereafter referred to as: the "test" population, the "same" population or group, or a deme member(s). In all cases these terms refer to the larger of the populations collected at a single granary trapping site. The

subjects trapped at the other granaries at the same location comprised the smaller populations and are referred to as: the other population or group, or an alien deme member(s).

A set of subjects, of two populations, was used for each of these replications of the basic experiment. Test subjects, four males and four females, were randomly chosen from the larger of the population groups. Two "same" group members were also randomly selected from this set of subjects and served as same deme stimulus odor sources. Likewise, two animals were chosen from the smaller population and provided the "other" odor stimuli.

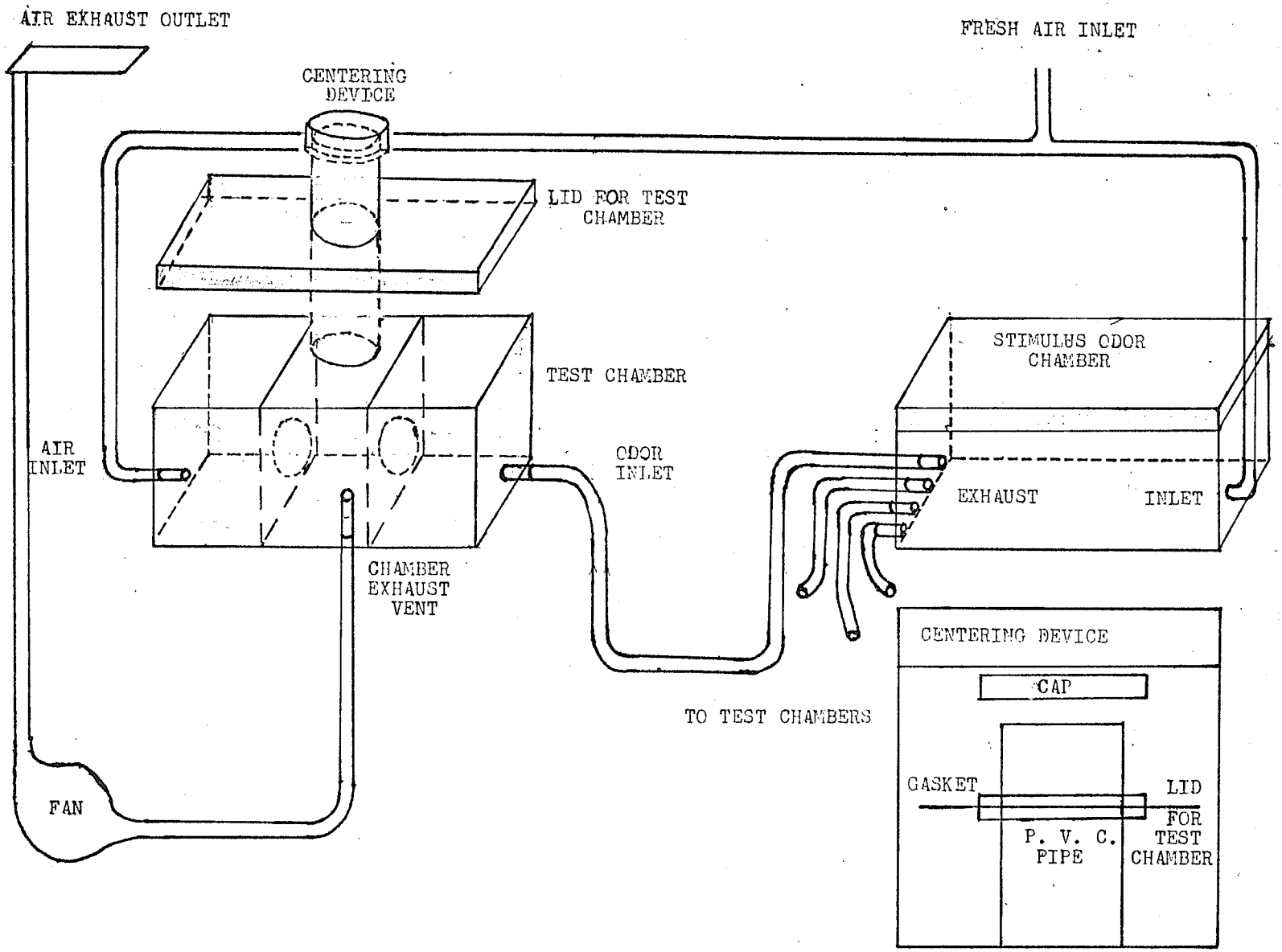
As was mentioned, the populations from which the "same" and "other" subjects came were of two sizes, a "larger" and a "smaller" population. This study was conducted in a year in which the mouse population was quite low. The general scarcity of mice in southern Manitoba at this time may in part explain an unusual aspect of the population studied. At each location which housed a population large enough to be used as a "test" subject population, a rather large (12 to 19 individuals) group of mice was found to inhabit one granary while nearby granaries were either uninhabited or contained but from two to five mice. An effort was made to determine if any of several factors could explain these differences. The size of the buildings, their relative protection from the wind, the possible use of pesticides and the past history of grain storage were considered. None of these factors proved helpful in explaining the population size discrepancies discussed. Therefore, when considering the follow-

ing series of experiments and their results several things should be kept in mind. First, the population structure of the subjects used here may not be normal and second, if this is the usual population pattern, some as yet unknown behavioral variable may be generating these discrepancies.

When the subjects were brought into captivity, each small social group or autonomous population was maintained in its own (separate) room for the duration of its captivity. The colony rooms did not share a common ventilation system. The mice were maintained on a diet of "Teklad" mouse and rat food. Food and water were always available in the home cage. The subjects were maintained on a 12:12 light-dark cycle with lights off at 1800 hours. All testing was conducted during the normal "awake" phase of the subjects' activity cycle.

Apparatus

Tests were conducted in 8 identical test chambers constructed of 16 gauge welded steel. Each test chamber measured 35.3 cm. x 10 cm. x 8.2 cm. and was divided into three equal compartments by a 16 gauge steel partition. Each compartment partition was pierced by a round hole 3.8 cm. in diameter, the lower edge of which was 2 cm. above the floor of the chamber, thus forming a hurdle. The floor of each end compartment formed a treadle. A lever attached to a rear corner of this treadle operated the timers used to record the amount of time a subject spent in each compartment. The apparatus is shown in Figure 1.


AIR EXHAUST OUTLET
FRESH AIR INLET
CENTERING DEVICE
LID FOR TEST CHAMBER
TEST CHAMBER
STIMULUS ODOR CHAMBER
AIR INLET
ODOR INLET
EXHAUST
INLET
CHAMBER EXHAUST VENT
TO TEST CHAMBERS
FAN
CENTERING DEVICE
CAP
GASKET
LID FOR TEST CHAMBER
P. V. C. PIPE

The chambers which housed the stimulus animals during the experiment were the same as the test chambers, except that the stimulus chambers were not divided into separate compartments. Also, the stimulus animal chambers did not have treadle-type floors. Both the test and stimulus animal compartments were fitted with air-tight metal (16 gauge) steel lids. Since such lids are also light-tight, all testing was done in a dark condition. Also, red lighting was used continuously in the testing room.

The problems of odor control led to the creation of an air transport system designed to assure proper air flow and maximally effective flow rates of the odor stimuli. Fresh air from a central compressed air source was filtered and forced at a regulated down-line pressure of 10 p.s.i. through a 3.8 cm. I.D. polyethylene pipe and into a stimulus animal compartment. The air and any odor stimuli then passed through 0.33 cm. I.D. (0.43 cm. O.D.) flexible plastic tubing and into one of the end compartments of a test chamber. Flexible tubing like that used for odor control was used to direct fresh air from a central distributor to the appropriate test chamber. Down-line pressure for the fresh air source was the same as that of the odor source.

The design of this experiment required that the various test odors be directed to a different test chamber and end compartment for each phase of the experiment. To facilitate the rapid coupling of the stimulus odor and fresh air hoses, a 0.43 cm. I.D. copper tube passed through the center of each extreme end

wall of each test chamber. This arrangement provided an air-tight, push-fit connection.

The air and any accompanying odor then circulated through the compartment into which it was introduced, through the interconnecting passageway to the center compartment where it was exhausted via a 0.43 cm. I.D. vent located in the center of the floor of the middle test compartment. The air was forced through 0.43 cm. I.D. plastic tubing and into a 3.8 cm. I.D. polyethylene pipe by a squirrel-cage type fan which directed the air and the odors it contained into the building's exhaust ventilation system.

In addition, the test rooms did not house any experimental animals while the study was in progress.

Design

Three sets of subjects were tested. Each group of test subjects was collected from a different area as described previously. Each group, or population of test subjects contained five male and five female mice. One male and one female from each population was randomly chosen as a stimulus animal for the test set. No test animal was ever used as a stimulus animal. Each test animal was permanently assigned to a test chamber which it alone occupied for all the tests within a set.

Each test subject was exposed to each of the four stimulus odor conditions. That is, a male and female from the same deme as the test subject and a male and female from another adjacent deme. All tests for a single group within Experiment 1 took place on the same day. That is, experiment 1 containing the

tests of three groups lasted three days. The order of the presentation of the series of stimulus odors was counterbalanced. This counterbalancing scheme is depicted in Table 1. In addition, the compartment (left or right) into which a stimulus was presented was also systematically counterbalanced. This counterbalancing scheme is shown in Table 2. All test animals were exposed to each odor for a 60 minute test session.

As has been indicated, there were three replications of the basic experiment. When the three replications were completed, a preliminary analysis was done to determine if replications should be included as a factor in the analysis. A 3 x 2 x 2 x 2 factorial analysis of variance with the third and fourth factors repeated was used as the major overall analysis. The factors were (a) replications; (b) test animal sex; (c) origin of group, the origin being either an animal from the same population as the test group , or an animal from an adjacent "other" population trapped at the same site and (d) stimulus animal sex.

When the initial analysis did not reveal significant differences between the three test groups (the analysis of variance for the first factor may be found in Appendix A) the data for the three replications were pooled for the analysis. Two separated analyses, one for time spent with the stimulus odor and one for time with the fresh air source were performed. This final analysis was a 2 x 2 x 2 factorial analysis of variance with the last two factors repeated. The factors were b, c, and d respectively (see above). This design meets the requirements cited by Kirk (1968) for the classic three factor split-plot

TABLE 1

Counterbalancing Scheme for Presentation of the Stimulus Odor

		Test Compartments			
		1, 5	2, 6	3, 7	4, 8
Trial	1	A	B	C	D
	2	B	C	D	A
	3	C	D	A	B
	4	D	A	B	C

TABLE 2

Counterbalancing Scheme for the Side of the Apparatus the Odor Stimuli were Presented on

		Test Subject (S)			
		1, 5	2, 6	3, 7	4, 8
Trial	1	Right	Left	Left	Right
	2	Left	Left	Right	Right
	3	Left	Right	Right	Left
	4	Right	Right	Left	Left

repeated measures design with two repeated factors. The Geisser and Greenhouse (1958) procedure was used to determine the F value with repeated measures. Their procedure is generally used to combat the problems of the assumption of homogeneity of the magnitude of the correlation between levels of the repeated factors inherent in this design. Multiple comparisons using Tukey's procedure (Kirk, 1968) were calculated for all main effects and interactions tested by the analysis of variance. The time spent near an odor condition, and the time spent in the vicinity of a fresh air (presumably neutral) condition were the dependent variables.

Procedure

Because this study is ultimately concerned with breeding patterns, only reproductively mature adults were tested. All male subjects' testes were scrotal. The estrous condition of the females was assured by injecting them daily with a single 10ug. subcutaneous dose of estradiol benzoate (EB) in 0.1 ml. of sesame oil. Following the fourth injection of EB, a 500ug. progesterone injection in 0.1 ml. of sesame oil was administered. All injections were administered at approximately 1400 hours. Testing was begun approximately four hours after the fifth (and each subsequent) EB injection. To confirm estrous, a vaginal smear was examined from all females at the time of the fifth injection. Vaginal smears were also examined from all test and stimulus females on the day they were to be used within the experiment.

The sudden placement of an animal into an unfamiliar enclosure

could possibly elicit an alarm or stress reaction. These reactions have been linked with urine-transported androgen metabolites whose odor can elicit a reaction in other individuals (Carr, Martorano & Krames, 1970). To minimize the chances of this occurring, a series of "familiarization trials" were conducted prior to testing. All animals were handled and allowed to explore the apparatus for 2 hours daily for 4 consecutive days before testing was begun. This included the stimulus or test compartment which each animal would occupy during testing. All the "familiarization trials" were conducted in a "fresh air" odor condition. Following a day's "familiarization trial", and after each actual test, the entire test apparatus was cleaned with a srong laboratory cleanser (Alconox) and water. The stimulus odor chambers were cleaned after each "familiarization trial" and following a day's test session. Whenever the subjects were handled, both during the "familiarization trial" and the actual testing, gloves were used. A separate pair of gloves was used for handling of each population tested.

Immediately prior to each test session the tubing which directed the odors and air flows to each of the test chamber compartments was coupled to meet the requirements of the test which was about to be conducted. A "white" masking noise was turned on. The air flow apparatus was activated and each test animal was transferred from its home cage to the centering device (see insert, Figure I) of the test chamber. The centering device was then raised and the test session begun.

The first odor condition was begun at 1800 hours. This

coincided with the normal active portion of the mouse day. Red lighting was provided during this period. All tests were 60 minutes in length. The inter-trial interval was approximately 45 minutes. This time was required to clean the test apparatus and adjust the air-flow equipment to meet the requirements of the next test. Following each test and during the inter-trial intervals, all animals were returned to their home cages where food and water were available. This was done to combat any possible fatigue or deprivation effects. This precaution did not eliminate the possibility of fatigue effects setting in during the later tests of a day. However, since the subjects are not required to perform a task, or "work", the probability of fatigue effects contaminating the data was felt to be small. Although the stimulus animals were removed from the stimulus odor compartments between trials, these compartments were not cleaned until after all the day's tests had been completed.

Results

When the three replications were completed, a preliminary analysis was done to determine if replications should be included as a factor in the analysis. A 3 x 2 x 2 x 2 factorial analysis of variance with the third and fourth factors repeated was used as the major overall analysis. The factors were (a) replications; (b) test animal sex; (c) origin of group, the origin being either an animal from the same population as the test group, or an animal from an adjacent "other" population trapped at the same site and (d) stimulus animal sex.

When the initial analysis did not reveal significant dif-

ferences between the three test groups (the analysis of variance for the first factor may be found in Appendix A) the data for the three replications were pooled for the analysis. Two separated analyses, one for time spent with the stimulus odor and one for time with the fresh air source were performed. The final analysis was a 2 x 2 x 2 factorial analysis of variance with the last two factors repeated.

Tables 3 and 4 summarize the results of the analysis of variance performed on the data of Experiment 1. The analysis indicated that for the time spent near an odor source measure (Table 3) the origin of group ($F = 196.85$, $df = 1/22$, $P < 0.001$) and stimulus animal sex ($F = 210.75$, $df = 1/22$, $P < 0.001$) main effects were significant. In addition, the test animal sex x origin of group ($f = 5.36$, $df = 1/22$, $P < 0.030$) interaction was siginificant. The analysis of variance for the other sources (test animal sex; test animal sex x stimulus animal sex; origin of group x stimulus and the test animal sex x origin of group x stimulus animal sex) did not reach significance at the 0.05 level.

The summary of the analysis of variance for the second measure, the time spent near the fresh air source (Table 4) showed that significant differences were found within the origin

TABLE 3

Analysis of Variance for Time Spent Near an Odor Source

Source	df	MS	F	P
Test Animal Sex	1	836823.00	3.29	0.083 (NS)
Subj. w. Groups	22	254068.63		
Origin of Group	1	33126736.00	196.85	<0.001
Test Animal Sex X Origin of Group	1	901904.00	5.36	0.030
Origin of Group X Subj.w. Groups	22	168280.75		
Stimulus Animal Sex	1	60710960.00	210.75	<0.001
Test Animal Sex X Stimulus Animal Sex	1	90098.00	0.31	0.582 (NS)
Stimulus Animal Sex X Subj. w. Groups	22	288066.86		
Origin of Group X Stimulus Animal Sex	1	736918.00	4.04	0.057 (NS)
Test Animal Sex X Origin of Group X Stimulus Animal Sex	1	435107.00	2.38	0.137 (NS)
Origin of Group X Stimulus Animal Sex Subj. w. Groups	22	182483.25		

TABLE 4

Analysis of Variance for Time Spent Near Fresh Air Source

Source	df	MS	F	P
Test Animal Sex	1	317515.00	2.86436	0.0105 (NS)
Subj. w. Groups	22	110850.19		
Origin of Group	1	25503808.00	136.95	<0.001
Test Animal Sex X Origin of Group	1	1247383.00	6.70	0.017
Origin of Group X Subj. w. Groups	22	186225.94		
Stimulus Animal Sex	1	42585328.00	192.56	<0.001
Test Animal Sex X Stimulus Animal Sex	1	105536.00	0.48	0.497 (NS)
Stimulus Animal Sex X Subj. w. Groups	22	221147.88		
Origin of Group X Stimulus Animal Sex	1	9643770.00	68.20	<0.001
Test Animal Sex X Origin of Group X Stimulus Animal Sex	1	1759589.00	12.44	0.002
Origin of Group X Stimulus Animal Sex X Subj. w. Groups	22	141395.31		

of the group ($F = 136.95$, $df = 1/22$, $P < 0.001$) and stimulus animal sex ($F = 192.56$, $df = 1/22$, $P < 0.001$) main effects, as well as the test animal sex x origin of group ($F = 6.70$, $df = 1/22$, $P = 0.017$), origin of group X stimulus animal sex ($F = 68.20$, $df = 1/22$, $P < 0.001$) and the test animal sex x origin of group x stimulus animal sex ($F = 12.44$, $df = 1/22$, $P = 0.002$) interactions. The other main effects and interactions for this measure were not significant ($P > 0.05$).

To clarify further the results of this experiment, each of these significant factors will be explored in greater detail below. In the case of interactions, these will be further elucidated with the results of Tukey's post-hoc ratio for mean comparisons Kirk, 1968). Generally, only those results significant at the 0.05 criterion level are reported. Also, although the two dependent measures were necessarily subjected to separate analyses, they do complement each other. Therefore, their analysis will be presented together. The reader's understanding of the main effects, their interactions and the comparisons within the interactions may be facilitated by reference to Tables 5 and 6 which display the cell means for odor and fresh air times respectively.

The origin of group effect reached significance for both independent measures. These results revealed that regardless of their own sex, or the stimulus animal's sex, the test subjects spent significantly more time near the odors of animals from their own group ($F = 196.85$, $df = 1/22$, $P < 0.001$). The fresh air analysis for this source was also significant ($F = 136.95$,

TABLE 5

Cell Means for Time Near an Odor Source

(in seconds)

Origin of Group	Stimulus Odor Stimulus Animal Sex	Test Animal Sex Female	Male	Marginals
Same Group	Male	1847.00	1270.50	1558.75
Same Group	Female	3066.33	2881.67	2974.00
Other Group	Male	168.42	248.92	208.67
Other Group	Female	2007.50	1941.25	1974.38
Marginals		1772.31	1585.58	1978.95

TABLE 6

Cell Means for Time Near Fresh Air Source

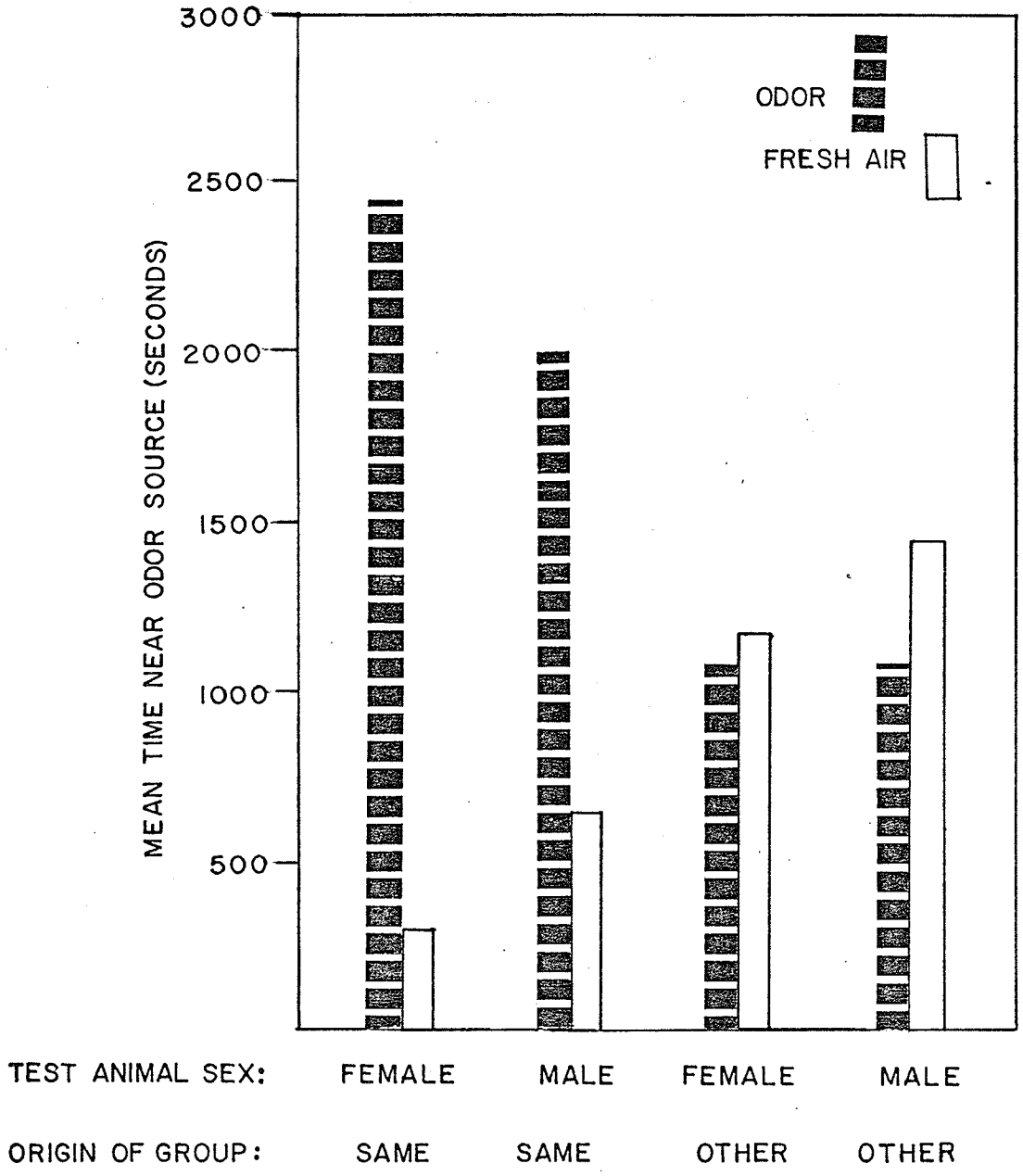
(in seconds)

Origin of Group	Stimulus Odor Stimulus Animal Sex	Test Animal Sex Female	Male	Marginals
Same Group	Male	493.83	1173.92	833.88
Same Group	Female	132.75	138.67	135.71
Other Group	Male	2657.33	2339.92	2498.63
Other Group	Female	486.92	578.42	532.67
Marginals		942.71	1057.73	1000.22

df = 1/22, P < 0.001). This analysis then revealed that not only did the mice used in this experiment prefer the odors of members of their own deme, but they actually avoided the odors of animals originating in a social group adjacent to their own. That is, these subjects spent more of their time in the apparatus near the fresh air source rather than near the odors of a member of another deme.

Not only did the subjects react differently to odor sources fron their own and another group, but the analysis of variance for both time near the odor source and time near the fresh air source (Tables 3 and 4) yielded significant results for the test animal sex x origin of group interactions (F = 5.36, df = 1/22, P = , 0.03, for odor time; F = 6.70, df = 1/22, P < 0.017, for fresh air). The post-hoc analysis of results helped identify further how subjects reacted to the odors of stimulus animals from their own or the other group. This analysis revealed that both males (q = 11/71, df = 1/22, P < 0.01) and females (q = 16.34, df = 1/22, P < 0.01) displayed a preference for the odors of their own group. In addition, both sexes spent more time near the fresh air source when given a choice between this odor source and that of another deme trapped in the same area (q = 9.12, df = 1/22, P < 0.001 for males; q = 14.29, df = 1/22, P < 0.01 for females). These effects can be seen in Figure 2.

Figure 2 also shows that both sexes spent the majority of the test period nearer the "same" odor source rather than near the fresh air source. The analysis further revealed that under this condition, males spent more time near the fresh air source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TEST ANIMAL SEX:
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ORIGIN OF GROUP:
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SAME
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(q = 13. 79, df = 1/22, P < 0.01). This comparison did not reach significance for the first dependent measure (q = 4.06, df = 1/22, P > 0.05). Along this same line, when confronted with the odors of an alien group, both male and female subjects spent more time near the fresh air source. These reactions can be seen in Figure 2. This multiple comparison also showed that females spent more of the test period avoiding the odors of other deme members than males did (q = 4.54, df = 1/22, P < 0.05).

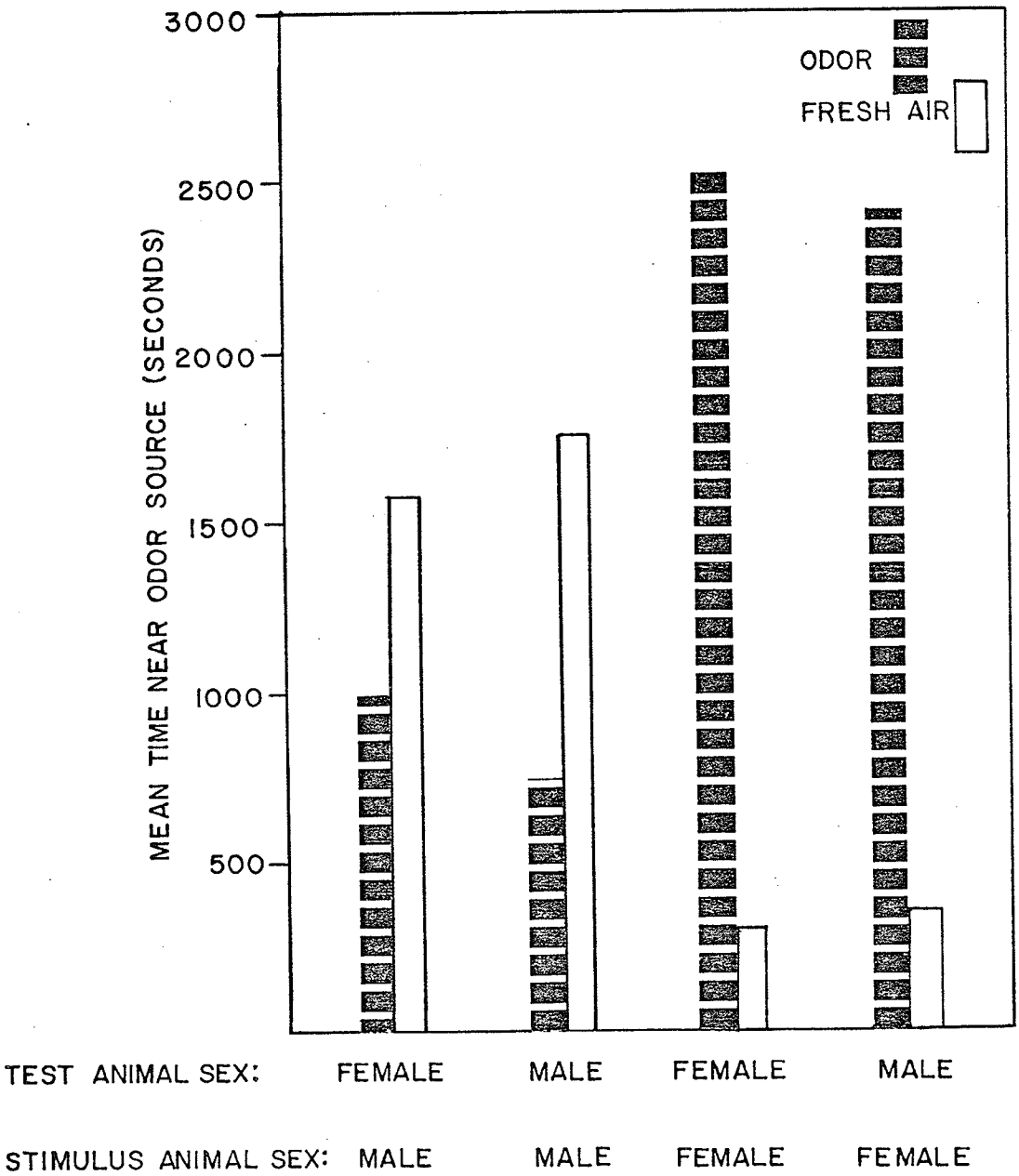
The major analysis revealed that stimulus animal sex was a significant factor for both measures. Briefly, these analyses (Tables 3 and 4) indicated that the odors of female stimulus animals were preferred over those of males (F = 210.75, df = 1/22, P < 0.001). The odors of males were also avoided more than female odors (F = 192.56, df = 1/22, P < 0.001). That is, more time was spent near a fresh air source than near a male's odor.

Given these results, it might be interesting to determine if test subjects respond differently by sex to the odors of male and female stimulus animals. However, the analysis of variance (Tables 3 and 4) indicated that the test animal sex x stimulus animal sex interaction did not approach significance for either measure. Therefore, caution should be exercised in accepting any significant results revealed by the following multiple comparisons. These results are, nonetheless, reported here because the major theoretical interest of this research was with finer grain contrasts, such as the male or female test subject's reaction to male or female stimulus animal's odors, rather than in combined differences as tested in the major analysis.

As was hinted at by the significant stimulus animal sex main effects reported earlier, these fine grain comparisons revealed that female stimulus odors were preferred by both test females ($q = 14.00$, $df = 1/22$, $P < 0.01$) and males ($q = 15.67$, $df = 1/22$, $P < 0.01$) over male odors. As might be expected, the inverse of this relationship was true for fresh air times. In other words, male subjects spent more time away from the odors of other males more than those of females ($q = 14.56$, $df = 1/22$, $P < 0.01$). The female subjects also avoided the odors of males more than females ($q = 13.18$, $df = 1/22$, $P < 0.01$). These results can be seen more clearly in Figure 3.

Finally, we might ask if test animals respond differently to the odors of males or female subjects on the basis of which group they represent. This origin of group x stimulus animal sex interaction (Tables 3 and 4) was shown to be significant for the fresh air time measure ($F = 68.20$, $df = 1/22$, $P < 0.001$), but did not quite reach significance for the time near the odor source measure ($F = 4.04$, $df = 1/22$, $P < 0.057$). Although multiple comparisons are introduced for both measures, the same caution as mentioned earlier is advanced for comparisons following the non-significant interaction.

As can be seen in Figure 4, the odors of females from the test subject's own deme were most highly preferred. This preference was significant when compared with the time a subject spent in the vacinity of the odors of a female from an alien group ($q = 11.69$, $df = 1/22$, $P < 0.01$), or males from the same group ($q = 14.29$, $df = 1/22$, $P < 0.01$). Both these odors were preferred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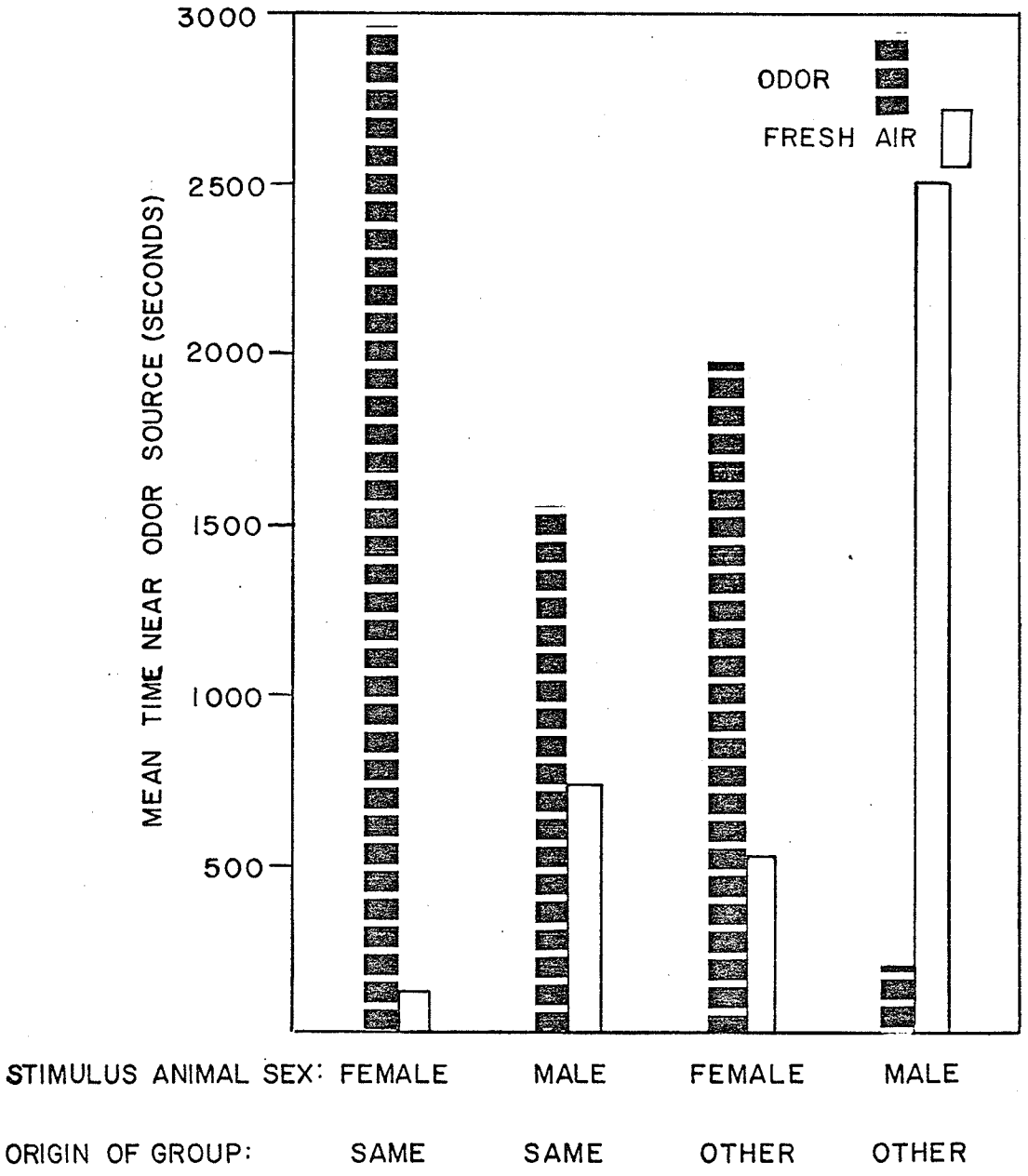
STIMULUS ANIMAL SEX: MALE MALE FEMALE FEMALE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ORIGIN OF GROUP: SAME SAME OTHER OTHER

over those of an alien male. More precisely, the odors of males from the same group as that of the test subject were more highly preferred than those of alien group females ($q = 17.83$, $df = 1/22$, $P < 0.01$).

The alien group male odors were avoided more than other stimulus odors. That is, test animals spent more time in the fresh air portion of the apparatus when the test odors came from foreign males than from males of the same group as themselves ($q = 20.26$, $df = 1/22$, $P < 0.01$) or alien females ($q = 22.62$, $df = 1/22$, $P < 0.01$). It should be pointed out here that this was the only choice combination in which the fresh air source was actually preferred over the odor source. These differences can also be seen in Figure 4. In addition, more time was spent avoiding the odors of males from the same deme as the subject than odors of females of that group ($q = 8.03$, $df = 1/22$, $P < 0.01$). The relationship between the time a subject spent in the odor portion of the apparatus as opposed to time in the fresh air area can be explored by referring to Figure 4.

EXPERIMENT 2

The finding that feral house mice prefer, or at least spend more time near, the odors of mice originating from the same deme as themselves and avoid the odors of adjacent deme members is interesting and suggests that olfactory processes may be important in reproductive isolation of subpopulation units. As mentioned earlier, a number of studies have demonstrated that small groups of mice, like demes, can remain separated from one another even though they share common home ranges or territorial boundaries (Anderson, 1964, 1967; Anderson and Hill, 1965; Reimer & Petras, 1967, 1968; Sealander, 1970). It is possible that the olfactory identity of either familiar "same" deme members, or mice belonging to another deme may mediate this group segregation process.

The isolation of these deme-type sub populations is generally believed to be maintained by a social system based upon the group defense of dominance areas or territories (Anderson & Hill, 1965, Reimer & Petras, 1967; Sealander, 1970). Defense of the territory is usually attributed to the males within the group. These individuals frequently patrol the boundary areas often marking them with odorous urine, feces or special glandular deposits (Anderson & Hill, 1965; Crowcroft, 1955; Crowcroft & Rowe, 1963; Eisenberg, 1962; Shillito, 1963; Thiessen, Blum & Lindzey, 1970). In addition, Lagerspetz (1964) demonstrated that male mice are inherently more aggressive than females. Therefore, if the home

range or territorial boundaries are maintained by some olfactory process, the odors of strange males would be avoided more than any other stimulus animal sex, origin of group stimulus odor combination. These results were obtained for Experiment 1.

In summary, the results of Experiment 1 indicated that house mice preferred the odors of members of their own group and avoided those of mice from an adjacent deme. This result suggests that olfaction could indeed mediate the continued segregation of smaller groups within the general population. In addition, the fact that the test subjects actively avoided the deme members who are thought to be the defenders of the between-deme boundaries (the males of an adjacent group) further supports this supposition.

If a social system based upon the group defense of dominance areas or territories is to be effective in inhibiting or preventing outsiders from breeding within the organized group its presence would have to be communicated to potential invaders. In fact, a territorial system would operate most efficiently if the borders of the prohibited area could be made "known" even if the "owner" was not there. Since odor is not only integral with the animal as it moves about engaging in its daily activities, but also remains after the animal has gone, it can serve as a long term reminder of the animal's passage. As has been mentioned, rodents may leave urine or fecal deposits in key places throughout their home ranges or territories as odorous signs of "ownership" available for scrutiny by passers-by (Eisenberg, 1962; Ralls, 1971). These marked areas may elicit intense

investigation by animals new to the area (Eisenberg, 1962,1963) or complete avoidance by individuals who have been defeated by the territory "owner" (Thiessen & Dawber, 1972).

It is possible that the individuals within side-by-side social groups, like demes, have been involved in aggressive confrontations. These confrontations were most likely integral with the establishment of the borders of the area(s) used exclusively by one or the other of the population subgroups. These groups' relative positions of area-related dominance and submission could easily have become associated with, and reinforced by, the odor cues unique to each population. It is reasonable then to expect that individuals will react differently when confronted with the odorous deposits left by either a member of their own group or a member of a neighboring unit. The purpose of Experiment 2 was to demonstrate that the odorous substances remaining in formerly occupied areas can be utilized by a mouse to determine if the previous occupant was a member of its own or an alien social group. In addition, Experiment 2 attempted to define the attraction, investigation or avoidance consequences of this detection.

Method

Subjects

The subjects for this experiment were the same as those used in Experiment 1. All maintenance procedures remained the same. The estrous condition of female subjects was maintained by the daily administration of EB injected subcutaneously at the same

times and in the same manner as described in Experiment 1. Vaginal smears were examined before the day's testing to confirm the estrous condition of the female subjects.

Apparatus

The apparatus consisted of a black, polyethylene coated plywood box, 50cm. x 50cm. x 25cm. This box was covered with a black plastic tarp. The tarp had several dozen pencil-sized holes for air entry. The only purpose of the tarp was to counteract the "corner effect" common to open field situations. A hardware cloth lid was fitted over the black tarp cover. The floor of this box was covered with clean sawdust to a depth of about 1.25 cm. A small grid, made of 2 cm. brass rods spaced 2 cm. apart measuring 10 cm. on a side, was placed in the center of the larger compartment. This small grid also rested on a sawdust area. However, the sawdust under the grid contained urine, feces and other debris from beneath the cage of one of the stimulus animals. The entire grid was connected to a body capacitance switch making the recording of the frequency of investigation and time in contact with the grid possible.

Design

A 3 x 2 x 2 x 2 factorial analysis with the last two factors repeated comprised the major analysis. The factors in this analysis were the same as those of Experiment 1. The replication factor can be found in Appendix 2. The Geisser and Greenhouse (1958) procedure for determining F values with repeated measures was again employed. However, in Experiment 2 the dependent measures consisted of (a) the time a subject was in contact with

the stimulus cage and (b) the number of discrete contacts the subject made with the stimulus cage.

As with Experiment 1, Experiment 2 was composed of three replications. Subjects of each replication were randomly assigned to either the stimulus or test condition. When the three replications were completed, the preliminary analysis was done to determine if replications should be included as a factor, collapsing across all other factors.

The data for the three replications were pooled when no significant effects were found in the preliminary analyses. Two major analyses, one for the time spent in contact with the odor source and one for the number of contacts with the odor source were performed. These analyses were of a 2 x 2 x 2 factorial type as described previously for Experiment 1.

Each population within a test set was composed of five male and five female subjects. One male and one female mouse from each of these populations was randomly assigned as a stimulus animal for the test set. Each subject was exposed to all four stimulus odor conditions. The series of stimulus odor presentation was counterbalanced as in Experiment 1. The counterbalancing scheme is depicted in Figure 2.

Test animals were exposed to each odor for a 10 minute test session. The intertrial interval was also 10 minutes. All tests within a single replication took place on the same day.

Procedure

All animals were confined within their home cages where food and water were continuously available. For a period of three

days prior to testing, a layer of clean sawdust, approximately 2.5 cm. deep, was in place beneath the cages. This sawdust area collected the urine, feces, and other debris which fell through the cages' hardware cloth floors. Thirty minutes prior to testing, the sawdust from below a stimulus animal's home cage was placed in a new paper bag containing approximately 153 cubic cm. of sawdust and was shaken for 60 seconds. The sawdust was then placed on the floor of the apparatus under the contact grid to a depth of about 1.25 cm. The urine and feces contained within the sawdust served as a stimulus odor. The remainder of the floor of the test apparatus was covered with clean sawdust to a similar depth. The contact grid was placed in the center of the large cubicle and the capacitance switch and recording equipment were connected to the contact grid. The "white" masking noise was then turned on.

A test animal was placed in the apparatus and the recording session was begun. A day's testing started at 1800 hours. The tests were run in the same order as in Experiment 1. A single replication was run on each of three days. Test animals were always introduced into a corner (the corner location was randomly varied) of the test cube. The subject was placed with its head facing the center of the cube. The number of contacts and the total time the test animal was in contact with the stimulus cage were recorded. All test sessions lasted 10 minutes. Following each test the subject was returned to its home cage for a 10 minute interval. At this time the contact grid was removed from the test cube and thoroughly cleaned with a strong laboratory for

the next test in the same manner as described above.

A habituation procedure was not used for Experiment 2 because it was felt that the potential for a test subject's learning cues other than olfaction associated with its exclusive use of an area was greater than the procedural danger of possible alarm reactions by a subject to a strange environment. Also, it was recognized that if a population unit contained both dominant and subordinate odor source animals a potential for confounding was present. However, this danger was minimized by the use of a design containg several replications. In addition, these experiments were attempting to test the reactions of the members of subpopulation units to odors representative of an alien groups' possessed area, regardless of individual dominance status.

Results

The analysis of variance for the first dependent variable, the time a test subject spent in contact with an odor source, is presented in Table 7. This table shows that the reactions to the origin of group ($F = 130.13$, $df = 1/22$, $P < 0.001$) and stimulus animal sex ($F = 208.97$, $df = 1/22$, $P < 0.001$) variables were significant. The following interactions also reached significance: test animal sex x origin of group ($F = 15.92$, $df = 1/22$, $P = 0.001$), test animal sex x stimulus animal sex ($F = 10.27$, $df = 1/22$, $P = 0.004$) and origin of group x stimulus animal sex ($F = 10.35$, $df = 1/22$, $P = 0.004$). The test animal sex main effect and the test animal sex x origin of group x stimulus animal sex three-way interaction were not significant at the 0.05 criterion

TABLE 7

Analysis of Variance for Time in Contact with an Odor Source

Source	df	MS	F	P
Test Animal Sex	1	0.94	0.00	0.986 (NS)
Subj. w. Groups	22	3010.06		
Origin of Group	1	295170.13	130.13	<0.001
Test Animal Sex X Origin of Group	1	36106.94	15.92	<0.001
Origin of Group X Subj.w. Groups	22	2268.21		
Stimulus Animal Sex	1	344639.94	208.97	<0.001
Test Animal Sex X Stimulus Animal Sex	1	16944.12	16.27	0.004
Stimulus Animal Sex X Subj. w. Groups	22	1649.22		
Origin of Group X Stimulus Animal Sex	1	20891.83	10.35	0.004
Test Animal Sex X Origin of Group X Stimulus Animal Sex	1	2.16	0.00	0.974 (NS)
Origin of Group X Stimulus Animal Sex X Subj. w. Groups	22	2018.52		

level.

These results are discussed in detail in the following paragraphs. However, since the two dependent measures of Experiment 2 are not related in the same manner as those of the previous experiment, they are discussed successively rather than simultaneously. Where appropriate, the interactions are more finely examined with Tukey's post-hoc ratio for mean comparisons (Kirk, 1968). Generally, only those results which reached significance at the 0.05 criterion level were reported. The cell means which comprised these relationships can be found in Table 8.

The major analysis as shown in Table 7 showed a significant origin of group effect ($F = 130.13$, $df = 1/22$, $P < 0.001$). Test subjects spent a greater proportion of the test period in contact with an area containing the odors of members of their own deme than with one housing the odors of another, adjacent deme.

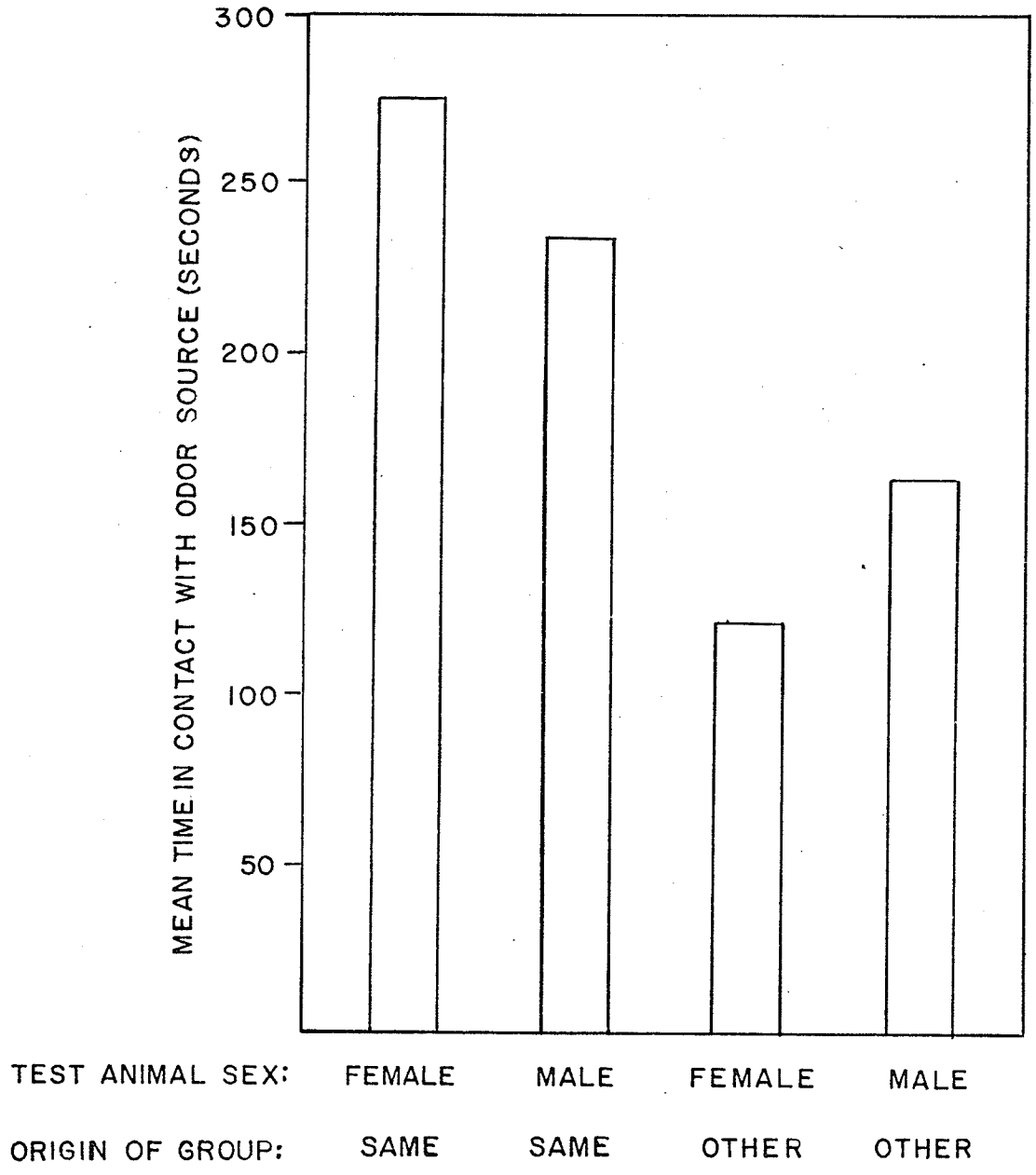
The significant test animal sex x origin of group interaction ($F = 15.92$, $df = 1/22$, $P = 0.001$) indicated that the time a subject was in contact with the odors of a group, as previously mentioned, was related to the sex of a subject. A closer look at this interaction, as shown in Figure 5, indicated that female subjects spent more time in contact with the odor areas of members of their own group than with those of the members of the other group ($q = 15.40$, $df = 1/22$, $P < 0.01$). Males were also in contact with the odors of members of the same deme longer than with the other odors ($q = 7.42$, $df = 1/22$, $P < 0.01$). Other comparisons within this analysis were not significant.

TABLE 8

Cell Means for Time Spent in Contact with An Odor Source

(in seconds)

Group of Origin	Stimulus Odor Stimulus Animal Sex	Sex of Test Subject Female	Male	Marginals
Same	Male	243.40	178.14	210.77
Same	Female	307.46	294.74	301.10
Other	Male	64.51	76.22	70.37
Other	Female	186.97	252.43	219.70
Marginals		200.58	200.38	200.48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MEAN TIME IN CONTACT WITH ODOR SOURCE (SECONDS)
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MALE
ORIGIN OF GROUP:
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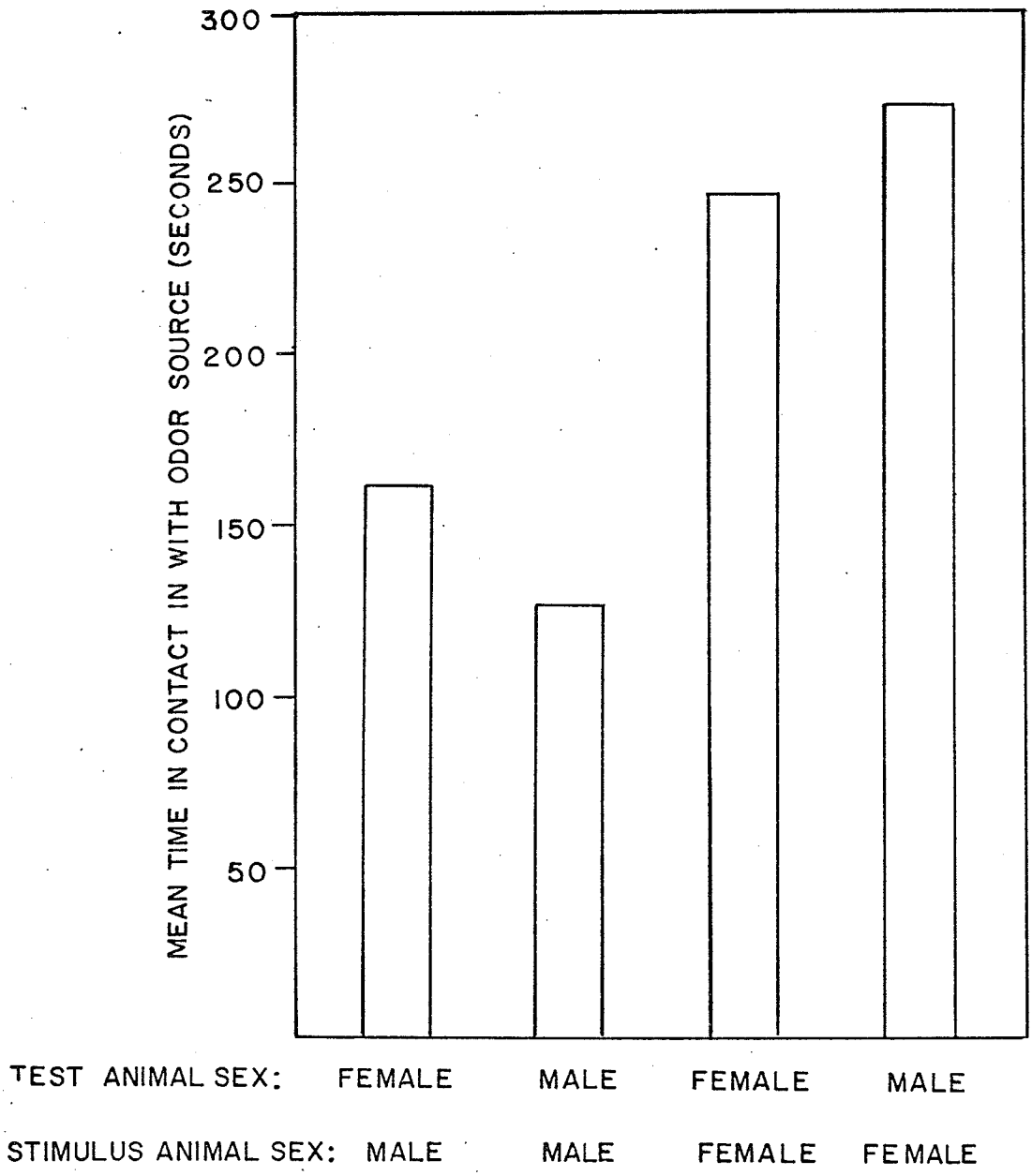
The stimulus animal's sex (Table 7) also significantly influenced the proportion of the test period the subjects were in contact with an odor source ($F = 208.97$, $df = 1/22$, $P < 0.001$). Regardless of the sex of the subject, or the group from which the stimulus animal originated, subjects spent more time in contact with the odors of female subjects than with males' odors.

In addition, the test animal sex x stimulus animal sex interaction showed that the sex of the subject was important in determining how long a mouse was in contact with either male or female stimulus odors ($F = 10.27$, $df = 1/22$, $P = 0.004$). Multiple comparisons indicated that females contacted the odor areas of stimulus females for a greater proportion of the test period than they did those of males (q 11.24, $df = 1/22$, $P < 0.01$). Male subjects also contacted those female odor areas for longer periods than the male stimulus odors ($q = 17.65$, $df = 1/22$, $P = < 0.01$). The other comparisons were non-significant. These results are shown in Figure 6.

Not only did test animals react differently to stimulus odors according to the sex of the donor, but also to the stimulus animals' origin. This is reflected in the significant origin of group x stimulus sex interaction reported in Table 7 ($F = 10.35$, $df = 1/22$, $P = 0.004$). This interaction was examined using the same post-hoc comparison as was used to analyze the other interactions. These fine-grained comparisons revealed that test subjects, regardless of sex, spent more time in contact with odors of females from their own deme than with those female mice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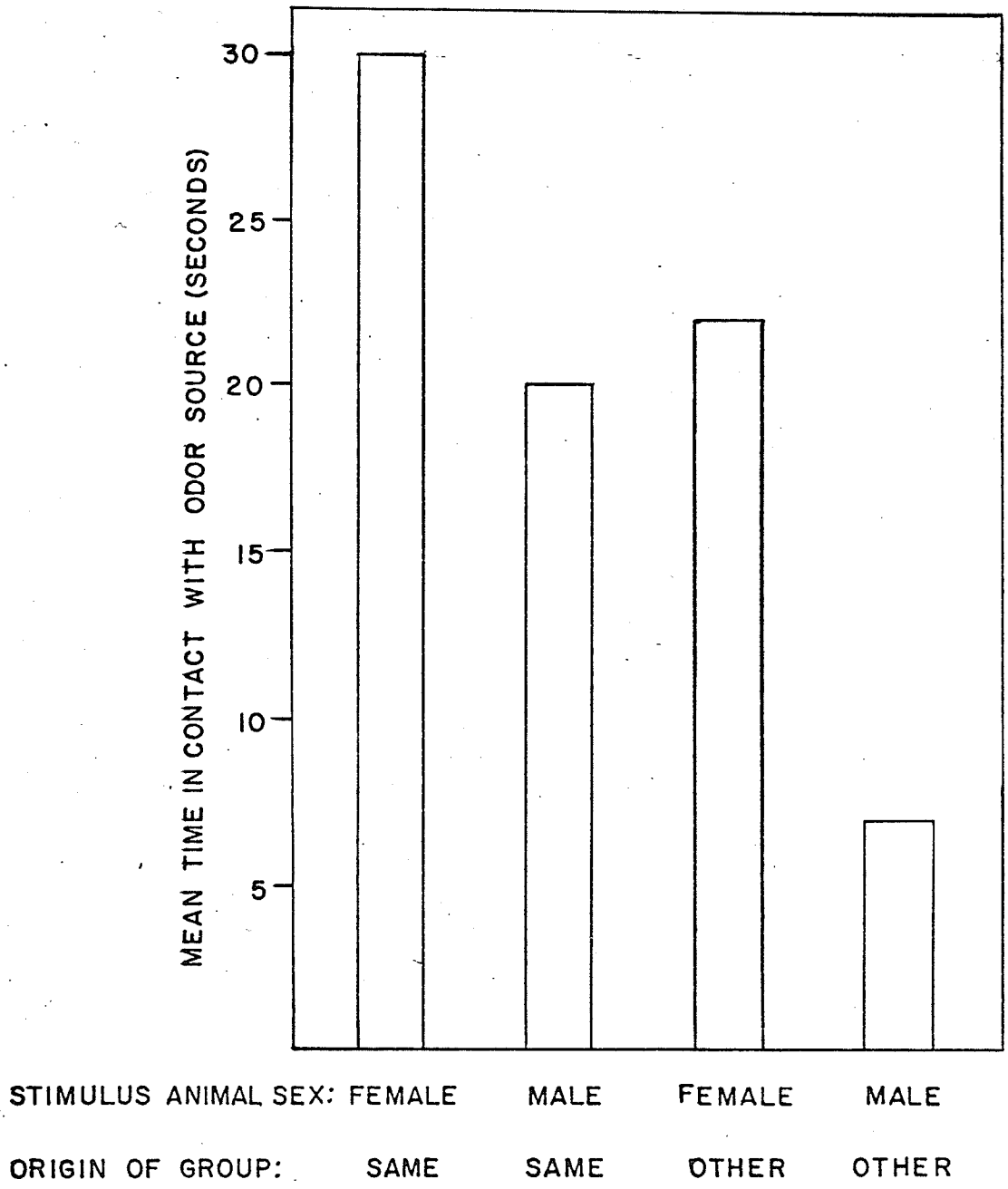
TEST ANIMAL SEX: FEMALE MALE FEMALE MALE
STIMULUS ANIMAL SEX: MALE MALE FEMALE FEMALE

from another adjacent social group ($q = 8.61$, $df = 1/22$, $P < 0.01$) or those of males from their own group ($q = 10.34$, $df = 1/22$, $P < 0.01$). In addition, subjects spent more time in contact with the odors of females from an alien deme than near those of males from that group ($q = 17.09$, $df = 1/22$, $P < 0.01$). Areas containing the odors of same deme males were contacted for more of the test period than were the odors of a donor male of another group ($q = 14.86$, $df = 1/22$, $P < 0.01$). Figure 7, which shows these comparisons, illustrates that subjects spent the most time in contact with the odors of females from the same deme as themselves, while alien male odors accumulated little contact time compared with other stimulus animal sex x group of origin odor combinations.

The analysis of variance of the second dependent variable, the frequency of contacts with an odor source, revealed that the test animal sex ($F = 20.50$, $df = 1/22$, $P < 0.001$) main effect was significant, as were the test animal sex x origin of group ($F = 26.76$, $df = 1/22$, $P < 0.001$), group of origin x stimulus animal sex ($F = 37.45$, $df = 1/22$, $P < 0.001$) and test animal sex x origin of group x stimulus animal sex ($F = 46.80$, $df = 1/22$, $P < 0.001$) interactions. These analyses, along with those which did not reach significance at the 0.05 confidence level, are summarized in Table 9. Cell means for these results can be found in Table 10.

As shown in Table 9, the test animal sex main effect was significant ($F = 20.50$, $df = 1/22$, $P < 0.001$). These results showed that male subjects contacted the odor source more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STIMULUS ANIMAL SEX: FEMALE MALE FEMALE MALE
ORIGIN OF GROUP: SAME SAME OTHER OTHER

TABLE 9

Analysis of Variance for Frequency of Contact with an Odor Source

Source	df	MS	F	P
Test Animal Sex	1	2656.50	20.50	<0.001
Subj. w. Groups	22	129.62		
Origin of Group	1	27.09	0.75	0.396 (NS)
Test Animal Sex X Origin of Group	1	969.01	26.76	<0.001
Origin of Group X Sub. w. Groups	22	36.21		
Stimulus Animal Sex	1	49.59	0.53	0.476 (NS)
Test Animal Sex X Stimulus Animal Sex	1	263.34	2.80	0.109 (NS)
Stimulus Animal Sex X Subj. w. Groups	22	94.13		
Origin of Group X Stimulus Animal Sex	1	1725.51	37.45	<0.001
Test Animal Sex X Origin of Group X Stimulus Animal Sex	1	2156.50	46.80	<0.001

TABLE 10

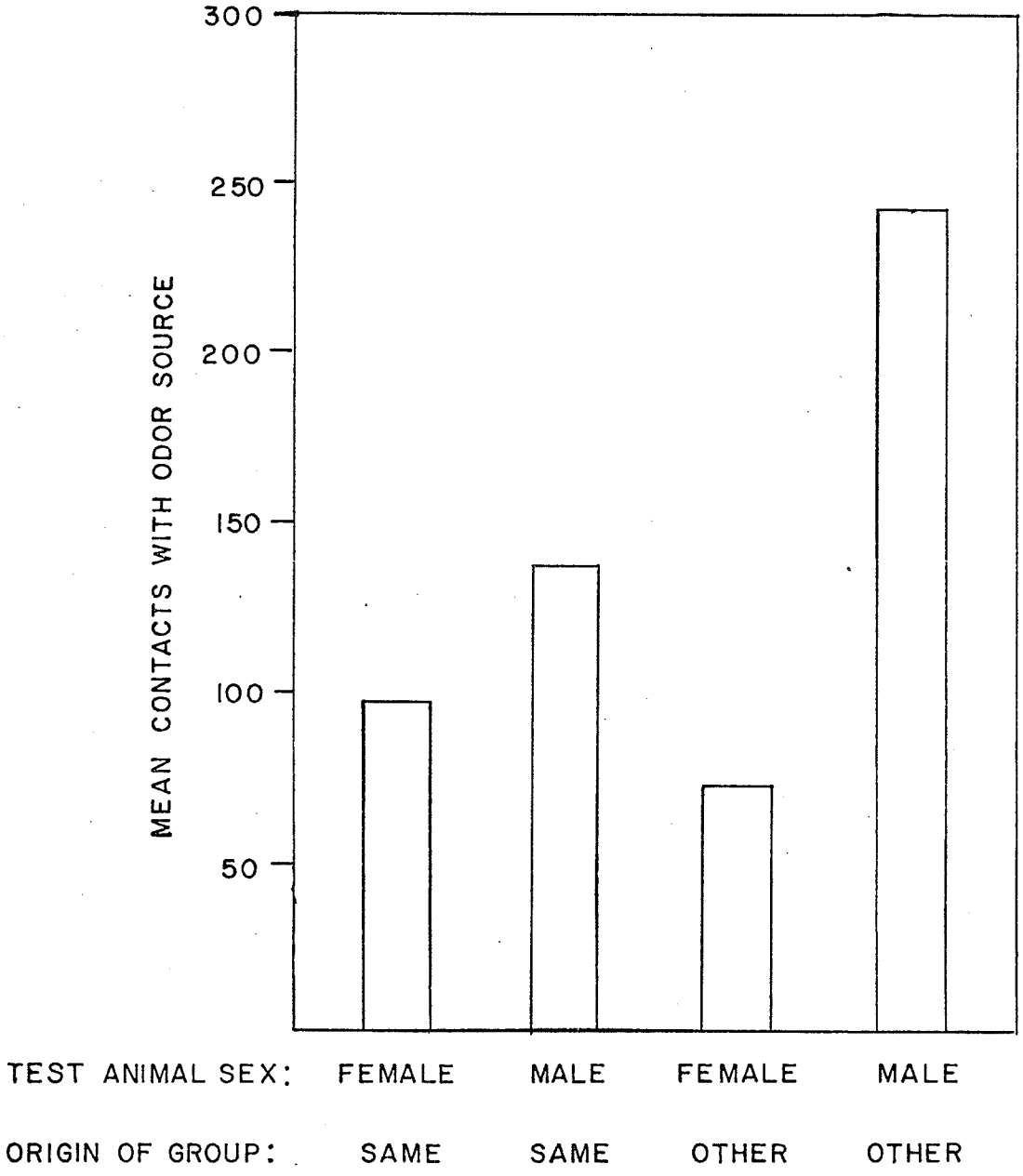
Cell Means for Frequency of Contact with An Odor Source

Group of Origin	Stimulus Odor Stimulus Animal Sex	Test of Sex Subject Female	Male	Marginals
Same	Male	12.83	10.83	11.83
Same	Female	16.58	26.92	21.75
Other	Male	4.42	34.08	19.25
Other	Female	10.17	14.25	12.21
Marginals		11.00	21.52	

frequently than did females. Male and female test subjects also reacted differently to the odors of stimulus animals from either their own, or another deme. This is reflected in the significant test animal sex x origin of group interaction seen in Table 9 ($F = 26.76$, $df = 1/22$, $P < 0.001$). When this relationship was examined more closely, the multiple comparisons indicated that when the odor area contained odor stimuli from members of a group other than that a test male belonged to, he contacted it more frequently than when the odors originated with a member of his own group ($q = 8.38$, $df = 1/22$, $P < 0.01$). Males also contacted the odors of these alien mice more frequently than females did ($q = 9.08$, $df = 1/22$, $P < 0.05$). These relationships can be seen in Figure 8. The other differences seen in this figure were nonsignificant.

Not only did male and female test animals react differently to the odors of members of their own or other groups, but regardless of sex the subjects also reacted differently to these odors when they originated with either male or female stimulus animals (origin of group x stimulus animal sex, $F = 37.45$, $df = 1/22$, $P < 0.001$). Multiple comparisons revealed that test subjects contacted the odors of females from their own groups more frequently than they did those of either males from the same deme as themselves ($q = 7.57$, $df = 1/22$, $P < 0.05$), or females from another adjacent deme ($q = 7.57$, $df = 1/22$, $P < 0.05$). These relationships are shown in Figure 9. Although nonsignificant ($q = 5.38$, $df = 1/22$, $P > 0.05$), the odors of males from an adjacent group were also contacted more frequently than either those of

males from the same group or females from the other group. Other differences displayed in this figure, but not reported here, did not reach significance at the 0.05 criterion level.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MEAN CONTACTS WITH ODOR SOURCE
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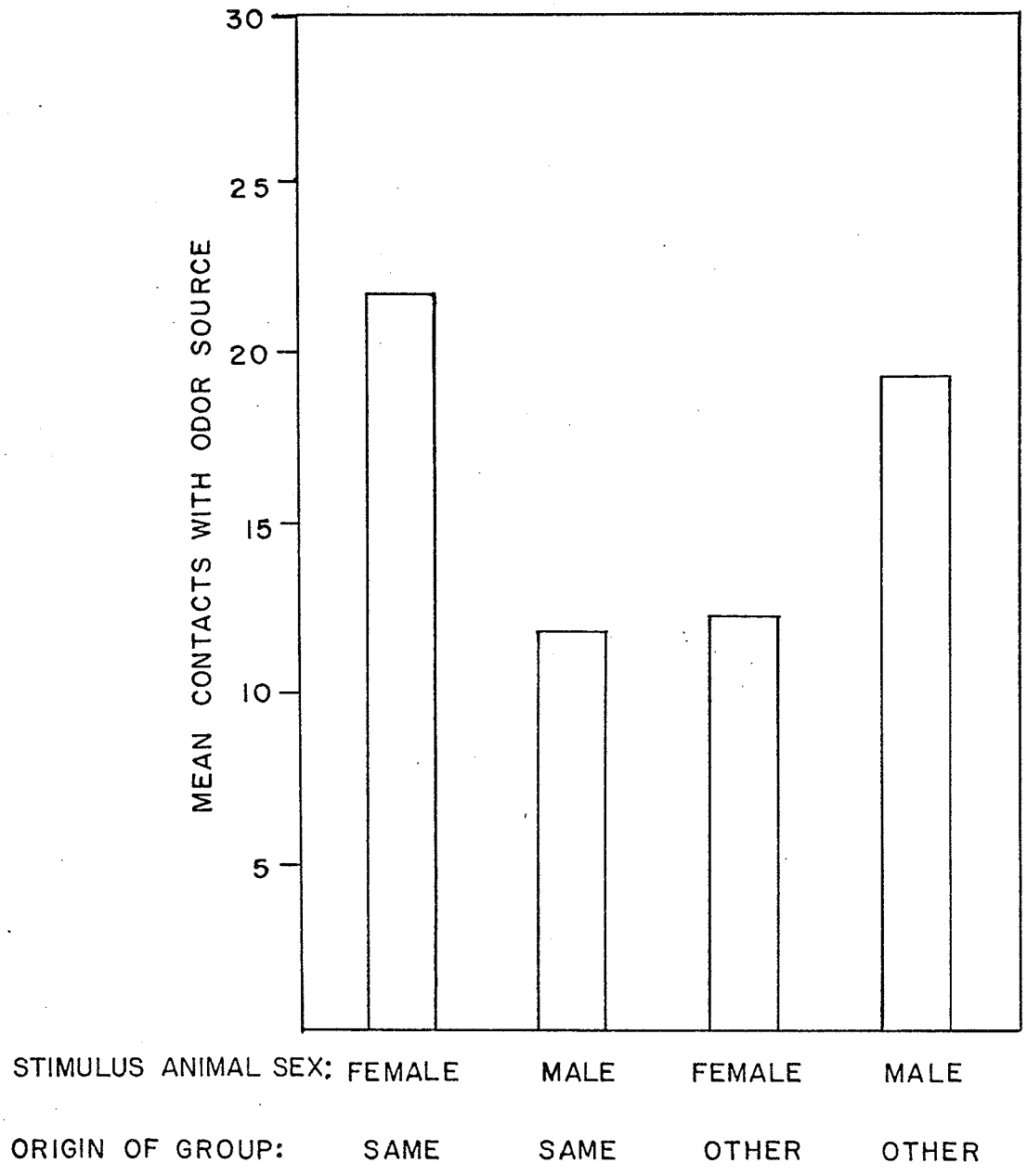
ORIGIN OF GROUP: SAME SAME OTHER OTHER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MEAN CONTACTS WITH ODOR SOURCE
STIMULUS ANIMAL SEX: FEMALE MALE FEMALE MALE
ORIGIN OF GROUP: SAME SAME OTHER OTHER

EXPERIMENT 3

So far the results of this experimental series have demonstrated that feral house mice respond differently to odors originating from their own or another deme. In the case of Experiment 1, these odors were air-transported while excretory products representing home ranges or territories comprised the odor source for Experiment 2. Specifically, Experiment 1 showed that mice spent more time in the vicinity of air transported odors originating with a member of their own deme than with the odors of mice from an adjacent but different group. These latter odors were also avoided. That is, more time was spent near a fresh air source than near them. These results indicated that it is possible for olfactory processes to mediate reproductive isolation between small, adjacent groups of mice.

This segregation process is commonly believed to be regulated by the group ownership of an area or territory (Anderson, 1964, 1967; Anderson & Hill, 1965; Reimer & Petras, 1967; Sealander, 1970). The boundaries of these territories are often marked in some manner by the owners (Desjardins, Maruniak & Bronson, 1973; Eisenberg, 1962, 1963; Ralls, 1971; Theissen & Dawber, 1972). Other mice are reluctant to cross these marked territorial boundaries (Reimer & Petras, 1967; Mackintosh, 1970). Experiment 2 was designed to assess differences in the reactions of mice to areas containing odorous materials associated with the animals (eg. territorial markers) as distinct from the animals them-

selves. The results demonstrated that differences did exist. Subjects spent more time in contact with an area containing the odors of the same deme than one containing the odors of less familiar mice. Johnson (1973) reported that males scent mark more frequently than females do. The results of Experiment 2 were consistent with this finding. Male mice contacted the odors of alien mice more frequently than they contacted areas with stimulus odors from mice of their own group. Eisenberg (1963) reported that urination spots in burrows were investigated thoroughly by intruders. The high frequency of male contact with alien odor areas reported here could be interpreted as being an example of this type of investigation. Eisenberg also noted that Heteromyid rodents marked areas previously marked by another animal. Ralls (1971) reported a number of similar results. Therefore, the high frequency of contact with alien odors by male mice could be associated with such marking. Ewer (1968) has argued that scent marking in a strange environment is the means by which a fearful animal "reassures" himself. Kleiman (1966) has argued that frequent urine marking may be evolutionarily derived from fear-induced urination.

It is clear that the high frequency of contact with alien odors probably does not constitute a preference for those odors. The results of Experiment 1 and the time measure of Experiment 2 indicate that more time is spent with familiar or same-deme odors. It appears that this is probably where the preference lies and that frequent contact with alien odors probably represents marking and/or investigation of the area. These results

lend support to the notion that olfactory identification may mediate reproductive isolation based on territorial defense.

Although these data suggest that olfactory cues may be involved in interdemetic reproductive isolation, they are nevertheless limited because a preference for an odor complex may bear little relation to the actual mating patterns of a population. Indeed, aside from the electrophoretical evidence reviewed previously, little evidence exists to suggest that isolation does occur at this level. The free ranging situation presents some obvious difficulties in monitoring social encounters and any assortative mating resulting from them. Thus, the problem can probably be studied best in the laboratory. Godfrey (1958) used a rather interesting technique for assessing relative sexual isolation. He allowed a male vole access to a group of females composed of voles of two species or subspecies.

A male's relative preference for mating with the representative groups of females was measured by merely noting a female's development of vaginal plugs, an indication of insemination. This technique may be easily adopted to the assessment of relative sexual isolation between population units.

The purpose of Experiment 3 was to determine if differences in the frequency of insemination occur when a male is allowed a choice between receptive females of two population units. In other words, Experiment 3 tested for the possibility of relative sexual isolation between demes.

Method

Subjects

The subjects used in this experiment were taken from the subject pool used in Experiments 1 and 2, and consisted of three adult males and twelve mature, estrous females. Six of the females were from the same populations as the males, and six were from populations in adjacent demes. All were maintained in the same manner as the subjects of the first two experiments except that the fur of the females from the "other" population were marked with a dye to facilitate identification.

Apparatus

The apparatus consisted of a black, polyurethane coated plywood box 50 cm. x 50 cm. x 25 cm. This box was covered with a hardware cloth lid and was partitioned into four equal compartments. The inside walls of each compartment were 24 cm. x 24 cm. x 25 cm. The space at the center of the cube where the partitions did not meet remained as a connecting passageway between each of the compartments. The floor of the apparatus was covered with about .25 cm. of sawdust.

Design

When this experiment was begun, it was found that the subjects reacted in a manner which had not been predicted. The subjects engaged in a great deal of aggressive behavior and mating seemed unlikely. Thus, the proposed dependent measure, the presence or absence of a vaginal plug, became inapplicable. In view of this,

an alternative measure was introduced.

As was mentioned, much aggression took place when the animals were released into a common space. Therefore, the frequency of aggressive bouts was used as the dependent measure. Aggressive bouts were scored as occurring either within a subject set or deme (same), or between subject sets, or demes (other). Only the more severe aggressive interactions consisting of striking with teeth, squealing when bitten and rolling and scratching (two animals) as described by Scott (1966) were recorded.

The original, planned experimental design can be found in Appendix B.

Procedure

To assure that the female subjects of Experiment 3 were in a sexually receptive condition, they received the same series of hormone injections as has been described for other experiments. Vaginal smears from female subjects were exmined on the day of testing to verify their estrous state. One hour after the last vaginal smear was taken, the estrous female subjects from each population were randomly assigned to a test compartment of the four-compartment chamber. A test male was then placed in another compartment and the trial begun. Thus, for each test session the subject composition was as follows: a single male; two females from the same deme; and two females from another, adjacent deme. The test animals were allowed to interact freely within the apparatus for 2 hours. The frequency of aggressive interactions between members belonging to a single deme were recorded as "same" aggression. Aggressive bouts between mice belonging to

different demes were recorded as "other" aggression. All subjects were then removed from the test apparatus and returned to their home cages.

Three replictions of this experiment were conducted. Each replication utilized new subjects. The apparatus was thoroughly cleaned with a strong laboratory cleanser (Alconox) and water and the sawdust floor covering renewed before each replication. As has been mentioned, the procedure described here is considerably different from that originally planned. The original procedure can be found in Appendix B.

Results

As has been noted, the measure employed in Experiment 3 was devised "on the spot" when other planned comparisons proved impossible to implement. This placed several restrictions on the data. As mentioned previouly, the composition of the test groups was unequal for both size and sex. That is, in each session there was a male and two females from one deme, and two females from another adjacent deme. Because only females from the adjacent deme were marked, aggressive interactions could only be scored as occurring between members within a single deme (same), or between members belonging to different demes (other). This combination could result in ten different interindividual aggressive interaction possibilites.

Six of these possibilities would be for "other" interactions and four for "same" interactions. Thus, if it is assumed that aggressive interactions are equally probable between all sub-

jects, "other" interactions would outnumber "same" interactions by a ratio of six-to-four. In other words, the "other" aggression scores were unavoidably inflated. In addition, the scores reflect both male-female and female-female interactions. The latter could not be considered as evidance for reproductive isolation. Because of these factors a statistical analysis could not be done. Therefore, only central tendency data is presented. Both mean and median frequencies for aggressive encounters between members of the same and other groups of mice are shown in Table 11. It is clear that, on the average, "other" aggressive interactions were over twice as frequent as "same" interactions. If the assumption of an equal probability of aggression is pursued, then the expected frequency would be 22. Since female mice are less likely to fight (Lagerspetz, 1964) most of the "other" observed aggression could be attributed to male-female interactions. Therefore, it seems reasonable to conclude that "other" aggessive interactions were indeed more frequent, and that they are consistant with the notion of reproductive isolation.

TABLE 11

Mean and Median Number of Aggressive Bouts

Between "Same" and "Other" Deme Members

Group	Male	Female	Median	Mean
Same	(N=3)	(N=6)	12	14.67
Other	(N=0)	(N=6)	31	34.00

EXPERIMENT 4

The possibility that territorial defence may be responsible for the reproductive isolation of potentially inbreeding populations of house mice has been discussed. The results of Experiments 1 and 2 lent support to this notion. That is, both these experiments demonstrated that mice spend more time near the odors of mice originating from their own deme, whereas they avoided the odors of mice of another adjacent deme. Experiment 1 utilized air transported stimuli while Experiment 2 used urine and feces, products often associated with territorial boundary marking. Experiment 3 was designed to determine if differences in the frequency of insemination occurred when a male was given a choice between females of his own group or another deme. Had a male bred with females of his own group rather than the "other" group females, a strong case for olfactorily mediated reproductive isolation would have been made. However, as has been noted, breeding did nct occur in the test situation described in Experiment 3. Instead, aggression occurred with considerable frequency. As a result of this, the dependent measure for Experiment 3 was changed from the freguency of insemination of "same" and "other" females (as evidenced by the presence of vaginal plugs) to the frequency of aggression between "same" and "other" group members. The results of Experiment 3 indicated that aggressive encounters with "other" group females were more than twice as frequent as aggressive bouts between "same" group

members. Although reproductive isolation was not illustrated by differences in the frequency of insemination between the two groups' members, the results of Experiment 3 suggested that reproductive isolation may occur between the groups in question. The high rate of aggression suggests that in the wild state receptive females would not breed with males from another deme. That is, in a situation in which the animals were not forced into confrontation (as the experimental design dictated) the "other" deme females would probably have avoided or escaped the encounter had they met the strange male at all. This supposition is supported by the results of Experiments 1 and 2 which showed that the odors of males from another deme were avoided. Also, in general, females spent very little time in contact with the odors of males.

Although most available evidence is based upon tests using male mice only, the evidence clearly indicates that aggression between even familiar mice can be expected when the odors of unfamiliar mice are present (Archer, 1968; Mugford and Nowell, 1970). Mackintosh and Grant (1966) reported results which indicated that the degree of unfamiliarity of a mouse's body odor, not the relative strangeness of the stimulus mouse was responsible for these aggressive exchanges. These responses may perhaps apply to any unfamiliar animal, regardless of sex. This is indicated by several findings. Reimer and Petras (1967) reported that female mice almost never become members of demes adjacent to their own. Also, Crowcroft and Rowe (1963) observed a complete absence of aggressive behavior within family grouping

of house mice. However, all members of the family group displayed aggression towards strange mice of either sex. In addition, Wolfe and Summerlin (1968) reported that when organized groups of cotton rats (Sigmodon hispidus) were housed with the members of unfamiliar groups general social disintegration and aggression were common occurences. Although not conclusive, such findings indicate that perhaps the results of Experiment 3 were not unusual. As was said, the high rate of aggression with "other" group females probably does reflect that sexual isolation would occur in a more natural situation.

The research reviewed above, and the results of Experiment 2 and 3 have a case for olfactorilly based interdemetic isolating mechanisms. Beyond these results we can ask if aggression at territorial boundaries and the subsequent avoidance of odors are a prerequisite for such isolation. In other words, is the interdemetic avoidance displayed in the first three experiments acquired by virtue of prior inter-deme interactions or by some other mechanism based upon mutual aversion to any "strange" odor.

Several studies have introduced evidence which indicates that many reactions are associated with prior aggressive or dominance related confrontations. For example, Bronson & Eleftheriou (1964, 1965) presented evidence indicating that mice subjected to defeat display increased activity of the pituitary-adrenocortical complex when exposed to the odor of the mouse who defeated them. Carr, Martorano, and Krames (1970) demonstrated that defeated mice prefer the odors of strange mice to those of the mouse who defeated them. In addition, Nyby, Thiessen and Wallace (1970)

showed that male gerbils (Meriones unguliculatus) marked an area less frequently when it had previously been marked by an animal who had defeated them. Also, Desjardins, Maruniak and Bronson (1973) found that while dominant male mice marked their entire cage, subordinates marked only its corners. This evidence then indicates that prior dominance associations are important in interindividual avoidance strategies.

On the other hand, it is possible that relatively strange odors, or odors which are not as familiar to a mouse as those of members of its own social group, elicit reactions which could be interpreted as interdemetic isolating mechanisms. A number of studies have demonstrated that strange mice are not readily integrated into established populations (Anderson, 1964, 1967; Anderson & Hill, 1965; Brown, 1953; Calhoun & Webb, 1953; Crowcroft & Rowe, 1963; Murray, 1967; Reimer & Petras, 1967; Wolfe & Summerlin, 1968). And, as is well known, aggressive interactions often occur when mice who are strangers to one another are placed together (Banks, 1962; Lloyd & Christian, 1967; Mugford & Nowell, 1970; Wolfe & Summerlin, 1968). In fact, Wolfe and Summerlin (1968) found that when groups of cotton rats (Sigmodon hispidus) trapped in areas several miles apart were placed together, more aggression and general social disintegration occurred than when the groups were trapped in the same area. Several authors have implicated oltactory variables in rodent aggression (Archer, 1968; Lee, 1970; Mugford & Nowell, 1970).

Therefore, we can ask if the differences obtained in Experiments 1, 2 and 3 can be attributed to the fact that the "other"

stimulus odors were less familiar than the "same" odor stimuli. Or, were the observed differences due to a kind of learning process? Since the probability of inter-deme aggression would be greatest at territorial boundaries, perhaps a mouse could associate this probability with the odors of individuals found there. If so, the members of alien groups would not be avoided, or would be avoided less, if these stimulus animals could not possibly have been known to the test animal beforehand.

Therefore, the purpose of the following series of studies was to determine the preference and avoidance consequences of subjects exposed to the odors of an unfamiliar mouse. This series of experiments did not define if the reaction to an odor complex was learned or unlearned. Nor did they help to define the process involved in acquiring the response. They did, however, help to determine if probable previous contact was a prerequisite for the response. In other words, does the odor of a mouse from another deme have consequences dependent on the proximity of that group to origin of the test animal?

Although the preceding experiments and those described in the following sections cannot be statistically compared, different outcomes suggest different possibilities. For example, if avoidance were based only on the basis of proximity and prior interaction, then avoidance of "other" odors would not be expected to occur in the following experiments. Or, if avoidance were based only on unfamiliarity (independent of prior interaction), then little difference would be expected between the results of the Experiment discussed previously and Experiment 4.

More specifically, Experiment 1 has established that house mice can differentiate between the odors of mice from their own or another group. Basically, the study demonstrated that mice prefer the odors of members of their own deme, especially females, while the odors of mice from another adjacent group are avoided. This avoidance was quite rigid when the stimulus odor was that of an adjacent group male. The "other" stimulus odors for that experiment originated from mice trapped but a few meters away from the test animals.

This experiment was almost identical to Experiment 1, except that Experiment 4 attempted to determine if familiar mice were still preferred when the "other" group odor originated with a totally unfamiliar mouse. In other words, could some sort of olfactorally based isolating mechanism exist between groups which had not previously acquired mutual avoidance type social habits? This then was the purpose of Experiment 4.

Method

Subjects

The test subjects for this experiment were the same as those used in Experiment 1. However, the "other" stimulus subjects used in Experiment 4 were not from populations immediately adjacent to the test population as was the case for Experiment 1. The stimulus subject populations were collected from locations geographically separated by several miles from the test subjects' origin. All subjects used in the experiment were aquired and maintained in the same manner as described in Experiment 1. In

addition, the estrous condition of the female subjects was maintained by the daily administration of EB injected subcutaneously at the same times and in the same manner as in Experiment 1.

Apparatus

The entire olfactory apparatus, including the test chambers, stimulus animal compartments and air transport system remained as described for the previous experiment.

Design

Experiment 4 utilized the same factorial analysis as did Experiment 1. The same counterbalancing schemes for both odor source and odor-fresh air presentation were also used. The only difference between this design and the previous one is that for this experiment the "other" stimulus animals were another, non-adjacent population trapped in a separate location rather than being trapped in the same area (group of farm buildings) as the test subjects. This then is a change in the composition of, but not the analysis of the repeated factor C. All other aspects of the design were the same as for Experiment 1.

Procedure

The general procedure used for Experiment 4 was previously described in Experiment 1.

Results

As in Experiment 1, the results for the time dependent variables, time near an odor source and time near a fresh air source, will be presented together for Experiment 4.

The major analysis results for the time a test subject spent near an odor source is shown in Table 12. This analysis of variance yielded significant main effects fot the test animal sex ($F = 21.17$, $df = 1/22$, $P < 0.001$), origin of group ($F = 321.09$, $df = 1/22$, $P < 0.001$) and stimulus animal sex ($F = 203.00$, $df = 1/22$, $P < 0.001$) variables. The test animal sex x origin of groups ($F = 15.94$, $df = 1/22$, $P < 0.001$), test animal sex x stimulus animal sex ($F = 8.29$, $df = 1/22$, $P = 0.009$) as well as the test animal sex x origin of group x stimulus animal sex ($F = 22.65$, $df = 1/22$, $P < 0.001$) interactions were all significant. The origin of group x stimulus animal sex interaction did not approach significance at the 0.05 criterion level. As with previous experiments, results which did not meet this criterion generally are not reported unless they facilitate understanding of the results. Also, as before, interactions were examined post-hoc with Tukey's ratio for mean comparisons. Cell means for these results are reported in Table 13.

Table 14 displays the analysis of variance for the time spent near a fresh air source. Here it can be seen that the origin of groups ($F = 112.01$, $df = 1/22$, $P < 0.001$) and stimulus animal sex ($F = 150.10$, $df = 1/22$, $P < 0.001$) main effects reached significance. The origin of group x stimulus animal sex ($F = 63.22$, $df = 1/22$, $P < 0.001$) interaction was the only interaction which reached significance for this measure. The cell means for all interactions of this analysis can be found in Table 15.

The significant test animal sex effect ($F = 21.17$, $df = 1/22$, $P < 0.001$) for the first dependent measure, the time spent near

TABLE 12

Analysis of Variance for Time Spent Near an Odor Source

Source	df	MS	F	P
Test Animal Sex	1	5104139.00	21.17	<0.001
Subj.w. Groups	22	241055.25		
Origin of Group	1	72658832.00	321.09	<0.001
Test Animal Sex X Origin of Group	1	3607601.00	15.94	<0.001
Origin of Group X Subj. w. Groups	22	226284.94		
Stimulus Animal Sex	1	320037376.00	203.64	<0.001
Test Animal Sex X Stimulus Animal Sex	1	1303390.00	8.29	0.009
Stimulus Animal Sex X Subj. w. Groups	22	157319.13		
Origin of Group X Stimulus Animal Sex	1	191887.00	1.23	0.280 (NS)
Test Animal Sex X Origin of Group X Stimulus Animal Sex	1	3535843.00	22.65	<0.001
Origin of Group X Stimulus Animal Sex X Subj. w. Groups	22	156105.31		

TABLE 13

Cell Means for Time Spent Near an Odor Souce

(in seconds)

Group of Origin	Stimulus Odor Stimulus Animal Sex	Sex of Test Subject Female	Male	Marginals
Same	Male	2831.83	1366.08	2098.96
Same	Female	3280.92	3048.92	3164.92
Other	Male	230.92	308.25	269.58
Other	Female	1626.50	1402.25	1514.38
Marginals		1992.54	1531.38	1761.96

TABLE 14

Analysis of Variance for Time Spent Near Fresh Air Source

Source	df	MS	F	P
Test Animal Sex	1	15402.00	0.05	0.827 (NS)
Subj. w. Groups	22	316587.31		
Origin of Group	1	50576032.00	112.01	<0.001
Test Animal Sex X Origin of Group	1	13920.00	0.03	0.862
Origin of Group X Subj. w. Groups	22	451496.13		
Stimulus Animal Sex	1	35948864.00	150.10	<0.001
Test Animal Sex X Stimulus Animal Sex	1	202952.00	0.85	0.367 (NS)
Stimulus Animal Sex X Subj. w. Groups	22	239503.93		
Origin of Group X Stimulus Animal Sex	1	20058608.00	63.22	<0.001
Test Animal Sex X Origin of Group X Stimulus Animal Sex	1	102835.00	0.33	0.575 (NS)
Origin of Group X Stimulus Animal Sex X Subj. w. Groups	22	317300.13		

TABLE 15

Cell Means for Time Spent Near the Fresh Air Source

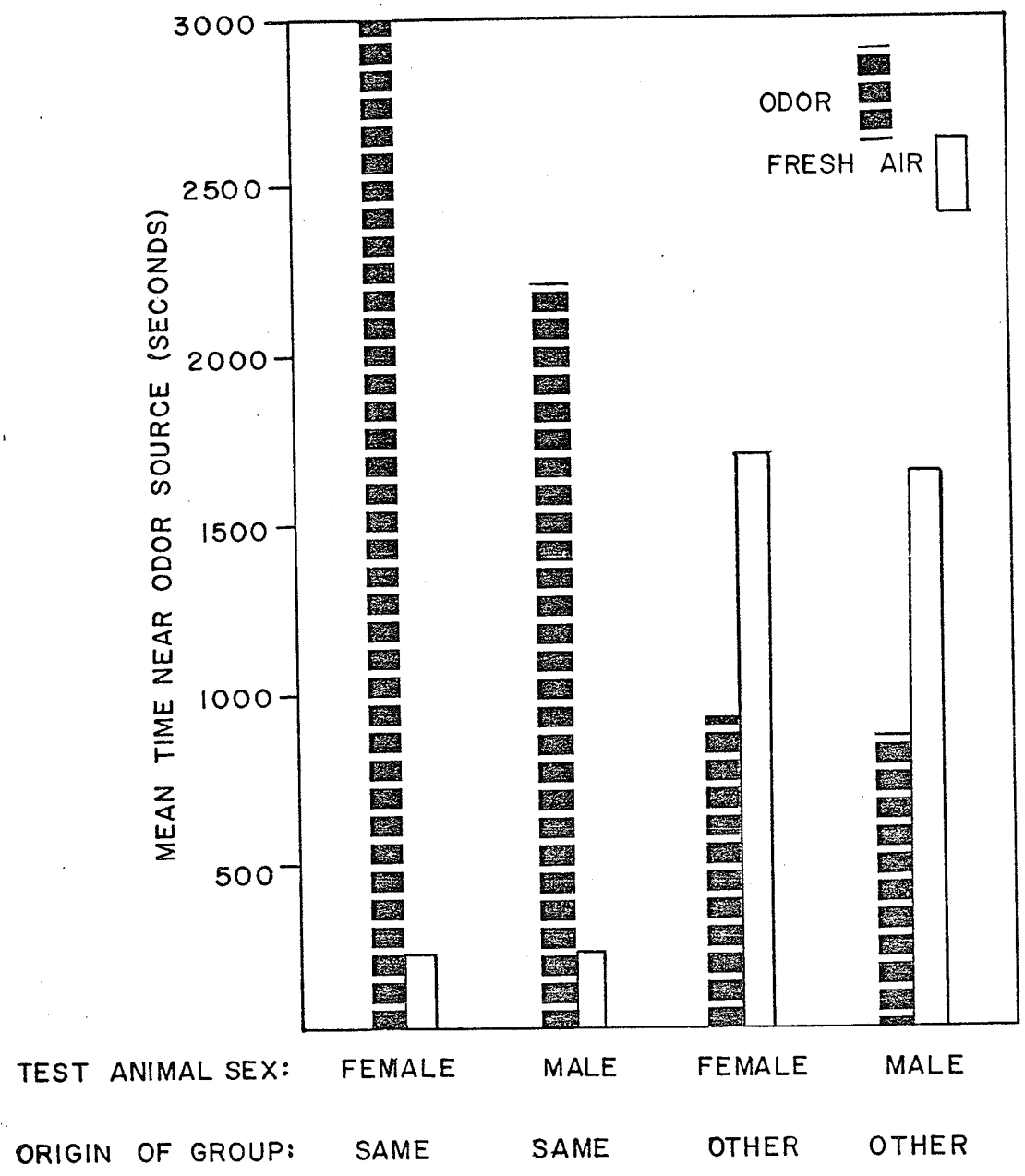
(in seconds)

Group of Origin	Stimulus Odor Stimulus Animal Sex	Sex of Test Subject Female	Male	Marginal
Same	Male	390.50	362.75	376.63
Same	Female	54.33	79.58	66.96
Other	Male	2845.92	2639.00	2742.50
Other	Female	550.42	658.42	604.42
Marginals		960.29	934.96	947.63

an odor source, indicated that female subjects spent more time near an odor source than males did. In addition, the significant origin of group effect ($F = 321.09$, $df = 1/22$, $P < 0.001$) suggested that regardless of sex, test mice spent a greater proportion of the test period near the odors of members of their own deme than those of a completely alien animal. This same main effect was also significant for the fresh air time measure ($F = 112.01$, $df = 1/22$, $P < 0.001$). It indicated that the odors of strange mice were avoided. That is, mice spent more time in the vicinity of the fresh air source when given a choice between that and the odor of another, unfamiliar mouse ($F = 112.01$, $df = 1/22$, $P < 0.001$).

The combination of these findings, the test subject x origin of group interaction, was significant ($F = 15.94$, $df = 1/22$, $P < 0.001$) for the time spent near an odor source. When this result was looked at in more detail (Figure 10) it was found that both male and female subjects spent more time near the odors of members of their own deme than near those of unfamiliar animals ($q = 16.70$, $df = 1/22$, $P < 0.01$, for males; $q = 26.28$, $df = 1/22$, $P < 0.01$, for females). Female subjects also spent more time neat the odors of members of their own group than males did ($q = 8.61$, $df = 1/22$, $P < 0.01$).

Figure 10 also shows that neither sex spent much time avoiding the odors of members of their own deme, whereas a great deal of fresh air time was accumulated when the choice was between the odors of strangers. Although the test animal sex x origin of group interaction did not reach significance ($F = 0.03$,


3000
2500
2000
1500
1000
500
MEAN TIME NEAR ODOR SOURCE (SECONDS)
ODOR
FRESH AIR
TEST ANIMAL SEX: FEMALE MALE FEMALE MALE
ORIGIN OF GROUP: SAME SAME OTHER OTHER

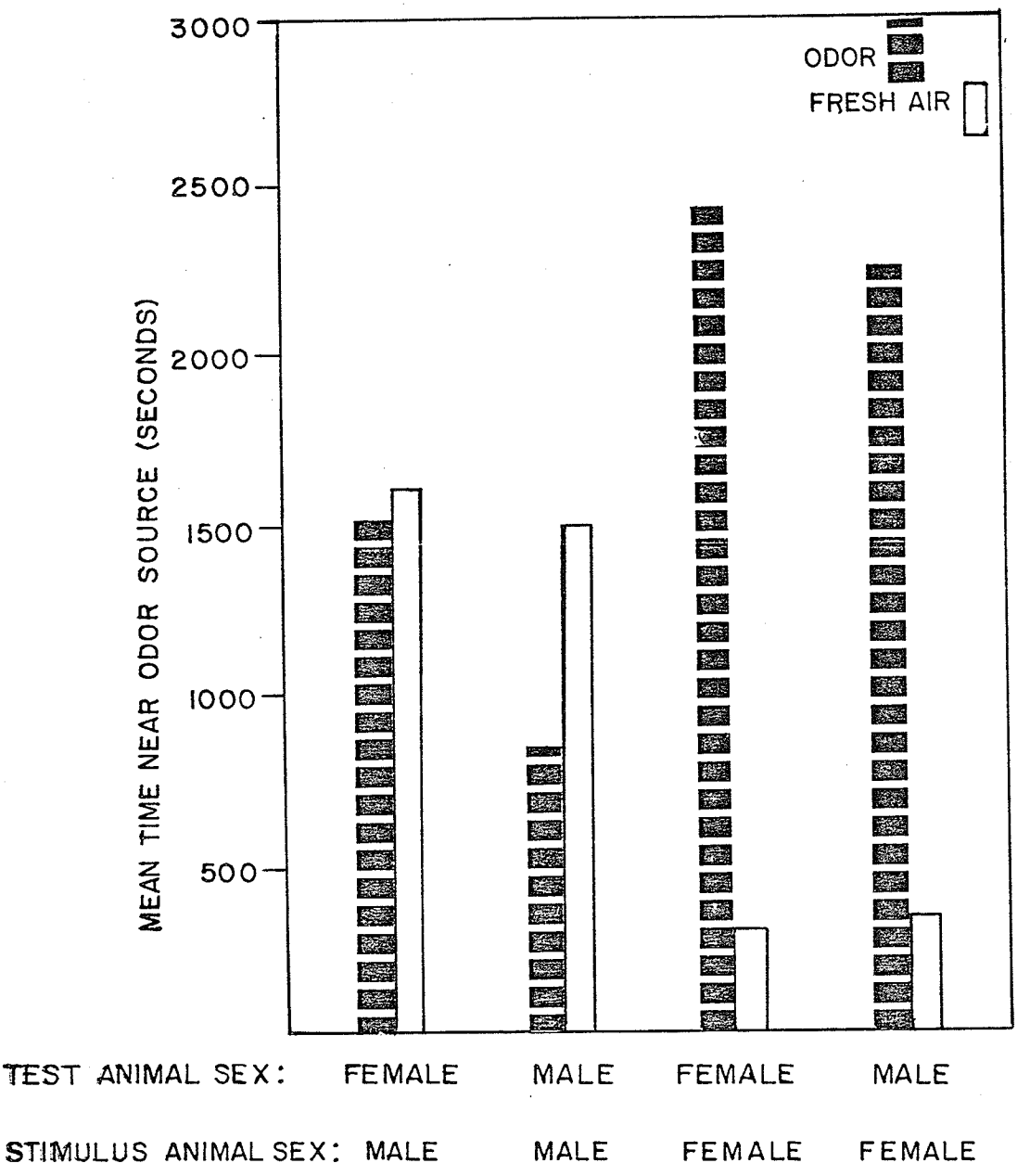
df = 1/22, P = 0.862) for the fresh air measure, a post-hoc comparison was performed because these finer comparisons were of greater theoretical interest than the combined differences tested by the main analysis. However, caution must be used when interpreting the results of multiple comparisons performed on non-significant interactions. This analysis then, revealed that both male and female test subjects avoided the odors of unfamiliar mice significantly more than they did the odors of mice from the same deme as themselves ($q = 10.41$, $df = 1/22$, $P < 0.01$, for males; $q = 10.76$, $df = 1/22$, $P < 0.01$, for females). That is, they spent more time near the fresh air source than near the strange mouse odor. Other differences displayed in Figure 10 did not reach significance at the criterion level.

The stimulus animal sex factor (Tables 12 and 14) was significant for both the time spent in the vicinity of both the odor source ($F = 203.64$, $df = 1/22$, $P < 0.001$) and the fresh air source ($F = 150.10$, $df = 1/22$, $P < 0.001$). The first result indicated that the odors of female stimulus animals were preferred over those of males regardless of the sex of the test subject or the origin of the stimulus odor. Conversely, the second finding suggested that the odors of males were avoided. That is, more time was spent on the fresh air side of the apparatus when the odors of a male were present than under a female odor condition.

The test animal sex x stimulus animal sex interaction (Tables 12 and 14) was significant for the time spent near the odor source ($F = 8.29$, $df = 1/22$, $P = 0.009$), but not for the time

near the fresh air source ($F = 0.85$, $df = 1/22$, $P = 0.367$). As with the previous non-sigificant interaction, these results were submitted to the post-hoc analysis described there. However, the post-hoc analysis of nonsignificant results is a questionably procedure and may not be viewed as statistically conclusive. As can be seen in Figure 11, subjects of both sexes spent more time in the vicinity of female odors than male odors ($q = 14.30$, $df = 1/22$, $P < 0.01$, for male subjects; $q = 9.50$, $df = 1/22$, $P < 0.01$, for females). Just the opposite trend is shown for the time a subject spent avoiding an odor. That is, male subjects spent more time in the fresh air compartment when the choice was between this and the odors of another male than they did when the choice involved a female's odor ($q = 11.32$, $df = 1/22$, $P < 0.01$). In addition, female subjects spent more time near the odors of male stimulus animals than males did ($q = 7.04$, $df = 1/22$, $P < 0.05$). Other comparisons were not significant.

Not only did test animals react differently to stimulus odors according to the sex of the donor, but Figure 12 suggests that this difference was also related to the stimulus animal's origin. This is the origin of group x stimulus animal sex interaction described in Tables 12 and 14. The major analysis of the time near the odor source measure for this interaction did not yield significant results ($F = 1.23$, $df = 1/22$, $P = 0.280$). As with other non-significant interactions, this one was also submitted to a post-hoc analysis. Again, caution must be exercised when accepting these results. The fresh air analysis for this interaction was significant ($F = 62.22$, $df = 1/22$, $P < 0.001$).


3000
2500
2000
1500
1000
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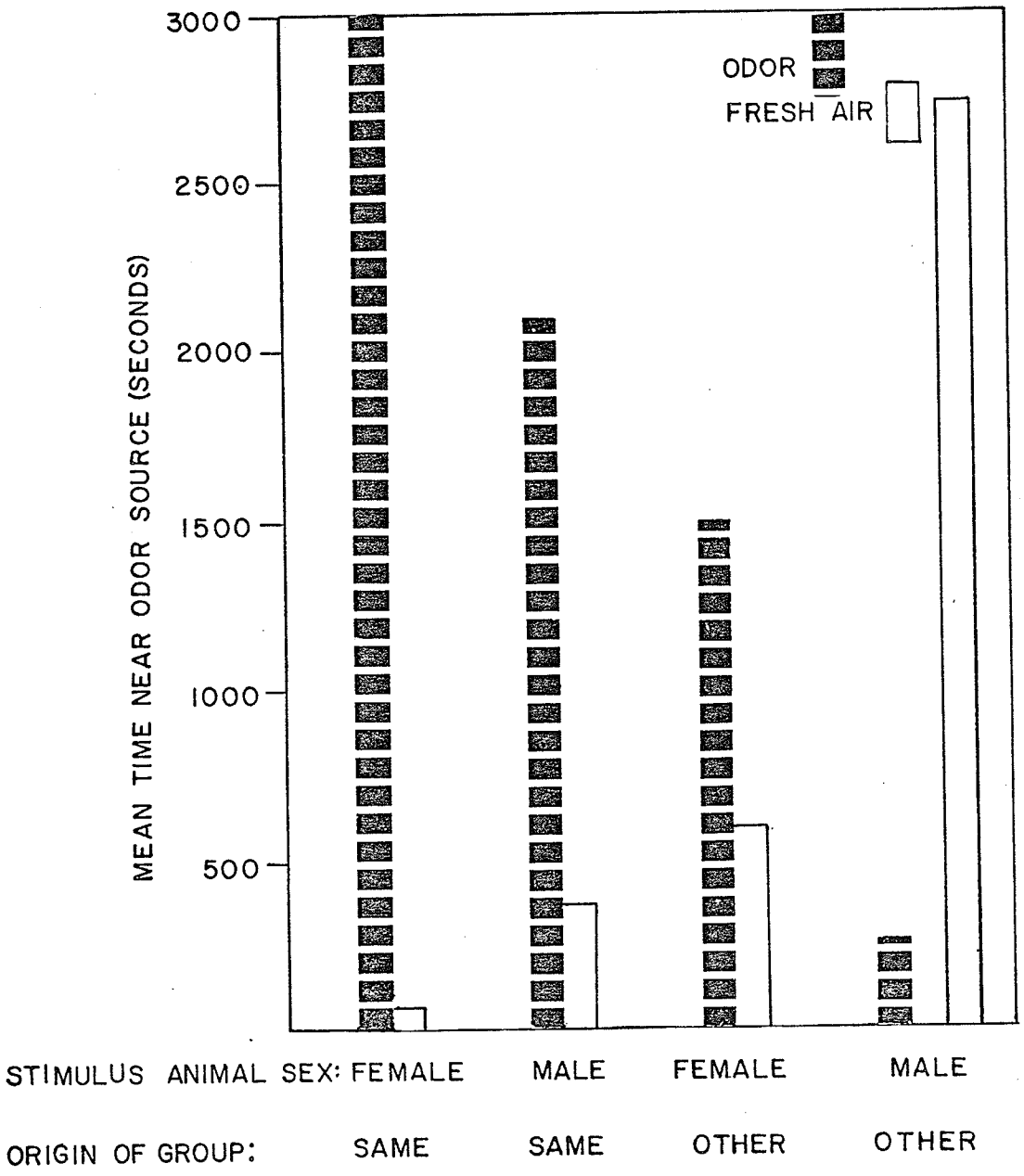
MEAN TIME NEAR ODOR SOURCE (SECONDS)
ODOR
FRESH AIR
TEST ANIMAL SEX: FEMALE MALE FEMALE MALE
STIMULUS ANIMAL SEX: MALE MALE FEMALE FEMALE


3000
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MEAN TIME NEAR ODOR SOURCE (SECONDS)
ODOR
FRESH AIR
STIMULUS ANIMAL SEX: FEMALE MALE FEMALE MALE
ORIGIN OF GROUP: SAME SAME OTHER OTHER

Inspection of Figure 12 indicates that the test subjects spent the greatest amount of time with the odors of female members of their own deme and the least time with alien male odors. A closer examination of these results reveal that the odors of females from the same deme as the test subject were preferred over those of males from that group ($q = 13.19$, $df = 1/22$, $P < 0.01$) and also over those of unfamiliar females ($q = 58.49$, $df = 1/22$, $P < 0.01$). More time was spent in the vicinity of odors of males from the same group ($q = 64.82$, $df = 1/22$, $P < 0.01$) and alien female odors ($q = 15.40$, $df = 1/22$, $P < 0.01$) than with the odors of unfamiliar males.

Not only were the odors of unfamiliar males preferred least, but more time was spent avoiding them than any other odor. Subjects remained in the fresh air compartment longer when the odor choice originated with an alien group male than with an unfamiliar female ($q = 19.85$, $df = 1/22$, $P < 0.01$), or a male from the test subject's own deme ($q = 18.69$, $df = 1/22$, $P < 0.01$). The odors of females from the same group as the subject elicited the least avoidance. Comparisons not discussed here did not reach significance at the 0.05 criterion level.

EXPERIMENT 5

The results of Experiment 4 were remarkably similar to those of Experiment 1. In both experiments, subjects spent significantly more time near the odors of members of another, strange deme. In addition, the odors of members of the alien deme were avoided more than those of same deme members. That is, subjects chose to spend their time near the fresh air source when an alien members odor was present in the opposite test compartment. The results of Experiment 4 indicate that prior experience with the odors of a neighboring deme is not necessary for that odor to be avoided. This suggests that the probable olfactorily mediated reproductive isolation discussed in relation to the preceeding experiments may be based upon reactions to unfamiliar or non-colony odors, rather than the association of a particular odor with known territorial boundaries.

The purpose of Experiment 5 was to determine if odors associated with territorial marking, such as urine and feces, would elicit the same patterns of contact time and frequency as were displayed in Experiment 2. If these patterns were the same, we could conclude that reactions to territorial markers do not require prior experience with that odor complex. However, results that differ from those of Experiment 2 would indicate that some kind of prior experience with those odors was required for between deme isolation to occur.

Method

Subjects

The test subjects used in Experiment 5 and their conditions of maintenance remained the same as has been described for the previous experiments. The major difference was that, as for Experiment 3, the "other" stimulus subjects were collected in locations other than those from which the test subjects came. Acquisition and maintenance of all subjects remained as described in Experiment 1.

All aspects of the apparatus remained the same as was described for Experiment 2.

Design

All aspects of the experimental design and data analysis for Experiment 3 remained the same as those of Experiment 2.

Procedure

The procedure used for Experiment 4 was the same as that previously described for Experiment 2.

Results

As with Experiment 2, the results for the time in contact and frequency of contact with an odor source are presented separately. The analysis of variance for the time in contact with the odor source dependent measure is presented in Table 16. The analysis indicated a significant main effect for the origin of group ($F = 194.16$, $df = 1/22$, $P < 0.001$) and the stimulus animal sex ($F = 225.47$, $df = 1/22$, $P < 0.001$) factors. In addition, the test animal sex x stimulus animal sex ($F = 29.34$, $df = 1/22$, $P <$

TABLE 16

Analysis of Variance for Time in Contact with an Odor Source

Source	df	MS	F	P
Test Animal Sex	1	3545.34	2.81	0.108 (NS)
Subj. w. Groups	22	1263.45		
Origin of Group	1	353005.75	194.16	< 0.001
Test Animal Sex X Origin of Group	1	27350.71	15.04	0.001
Origin of Group X Subj. w. Groups	22	1818.13		
Stimulus Animal Sex	1	496972.25	225.42	< 0.001
Test Animal Sex X Stimulus Animal Sex	1	64677.36	29.34	< 0.001
Stimulus Animal Sex Subj. w. Groups	22	2205.13		
Origin of Group X Stimulus Animal Sex	1	37849.88	20.55	< 0.001
Test Animal Sex X Origin of Group X Stimulus Animal Sex	1	2820.99	1.53	0.229 (NS)
Origin of Group X Stimulus Animal Sex X Subj. w. Groups	22	1841.48		

0.001) and the origin of group x stimulus animal sex ($F = 20.55$, $df = 1/22$, $P < 0.001$) interactions were significant. The analysis of variance for the other sources (test animal sex main effect and the test animal sex x origin of group x stimulus animal sex three-way interaction) did not reach significance at the 0.05 criterion level.

These significant factors will be explored in greater detail below. Interactions are further elucidated with the results of Tukey's post-hoc ratio for mean comparisons (Kirk, 1968). In general, only those results which reached significance at the 0.05 criterion level are reported. Table 17 shows the cell means for the time subjects spent in contact with an odor source.

As was said, the origin of group factor was significant. These results revealed that regardless of their own sex, or the stimulus animal's sex, the test subjects spent significantly more time in contact with the odor areas of members of their own deme ($F = 194.16$, $df = 1/22$, $P < 0.001$).

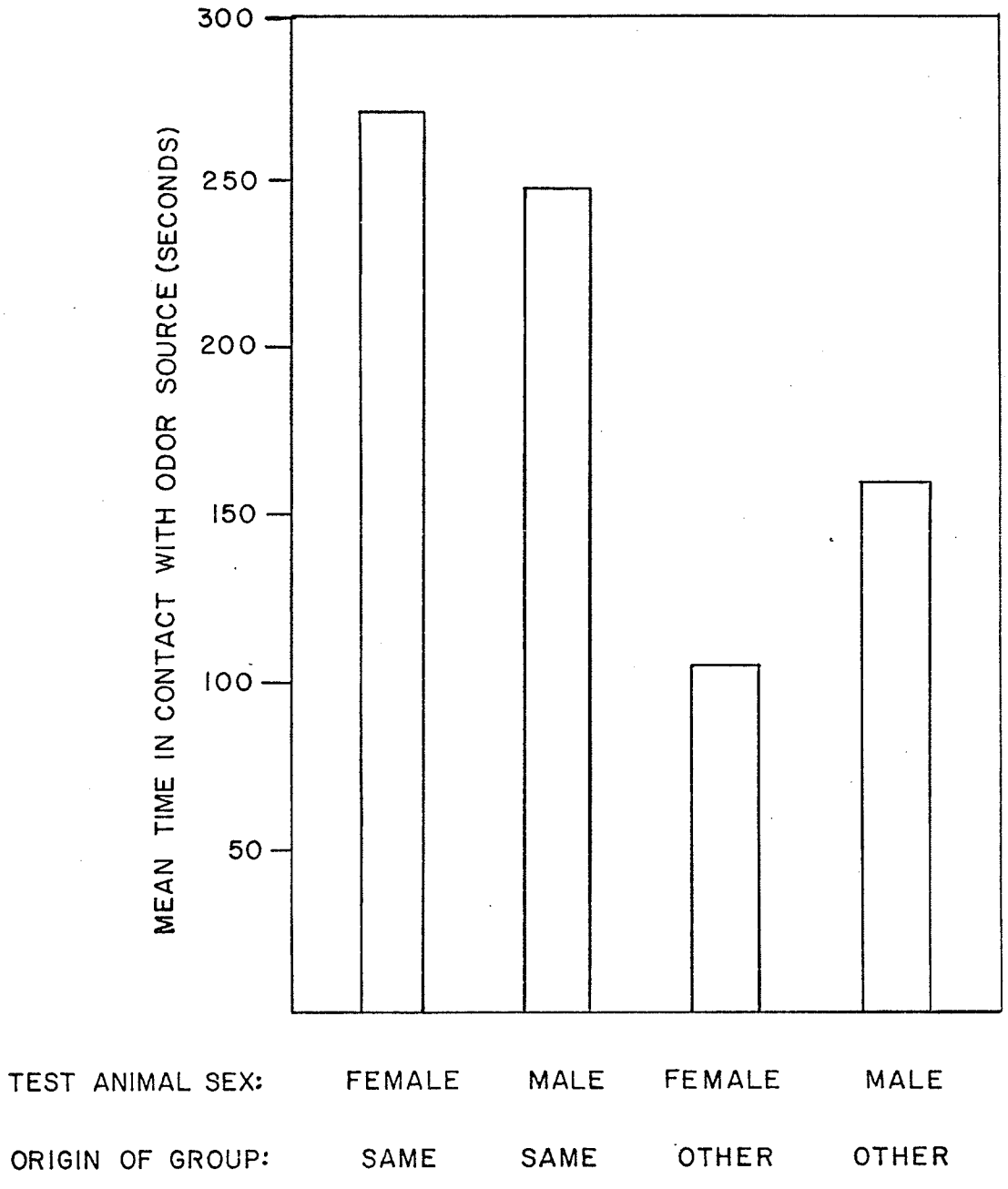
The analysis of variance (Table 16) revealed that this result contributed to a significant test animal sex x origin of group interaction ($F = 15.04$, $df = 1/22$, $P = 0.001$). A post-hoc analysis further identified subjects' reactions to the stimulus odors of animals from either their own or an unfamiliar group. As seen in Figure 13, both male and female subjects spent a greater proportion of the test period in contact with an area containing the odors of members of their own deme than with one bearing an unfamiliar deme's odors ($q = 17.82$, $df = 1/22$, $P < 0.001$, for females; $q = 10.60$, $df = 1/22$, $P < 0.001$, for males).

TABLE 17

Cell Means for Time in Contact with an Odor Source

(in seconds)

Origin of Group	Stimulus Odor Stimulus Animal Sex	Test Animal Sex Female	Male	Marginals
Same Group	Male	249.40	165.04	207.22
Same Group	Female	290.83	331.98	311.40
Other Group	Male	43.81	48.65	46.23
Other Group	Female	186.35	273.33	229.84
Marginals		192.60	204.75	198.67


300
250
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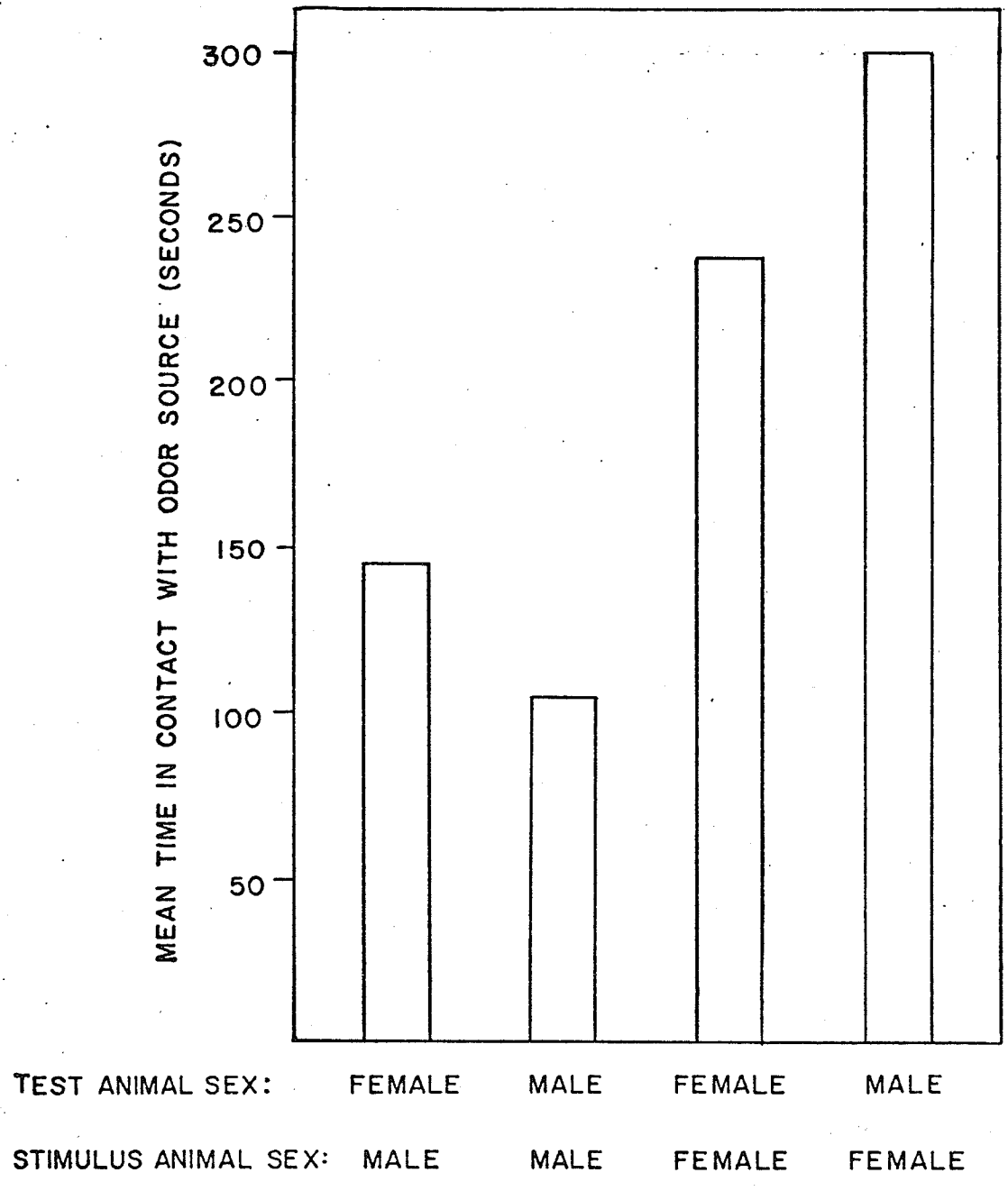
MEAN TIME IN CONTACT WITH ODOR SOURCE (SECONDS)
TEST ANIMAL SEX:
FEMALE
MALE
FEMALE
MALE
ORIGIN OF GROUP:
SAME
SAME
OTHER
OTHER

In addition, males contacted the unfamiliar deme's odors longer than females did ($q = 5.73$, $df = 1/22$, $P < 0.01$).

The major analysis also indicated that the stimulus animal sex factor was significant. Specifically, subjects spent more time contacting the areas which contained the odors of female stimulus animals than those housing male odors ($F = 225.47$, $df = 1/22$, $P < 0.001$).

This effect is reflected in the significant test animal sex x stimulus animal sex interaction ($F = 29.34$, $df = 1/22$, $P < 0.001$) reported in Table 16. Multiple comparisons indicated that areas containing the odors of a female stimulus animal were contacted for a greater proportion of the test period than those containing male odors. This was true for both male ($q = 20.44$, $df = 1/22$, $P < 0.01$) and female ($q = 9.60$, $df = 1/22$, $P < 0.01$) test animals. In addition, male test subjects spent more time contacting these females odors than did female subjects ($q = 7.54$, $df = 1/22$, $P < 0.05$). All other comparisons were nonsignificant (Figure 14).

A question which hinges upon the significant stimulus animal sex and group of origin main effects is how test subjects, regardless of sex, reacted to the odors of male or female stimulus animals based upon the group to which the stimulus animal belongs. This relationship is reflected in the significant origin of group x stimulus animal sex interaction seen in Table 16 ($F = 20.55$, $df = 1/22$, $P < 0.001$). This interaction, and the relationship it represents is depicted in Figure 15. This figure and post-hoc analysis revealed that in general the test subjects spent more time in contact with the odors of female


MEAN TIME IN CONTACT WITH ODOR SOURCE (SECONDS)
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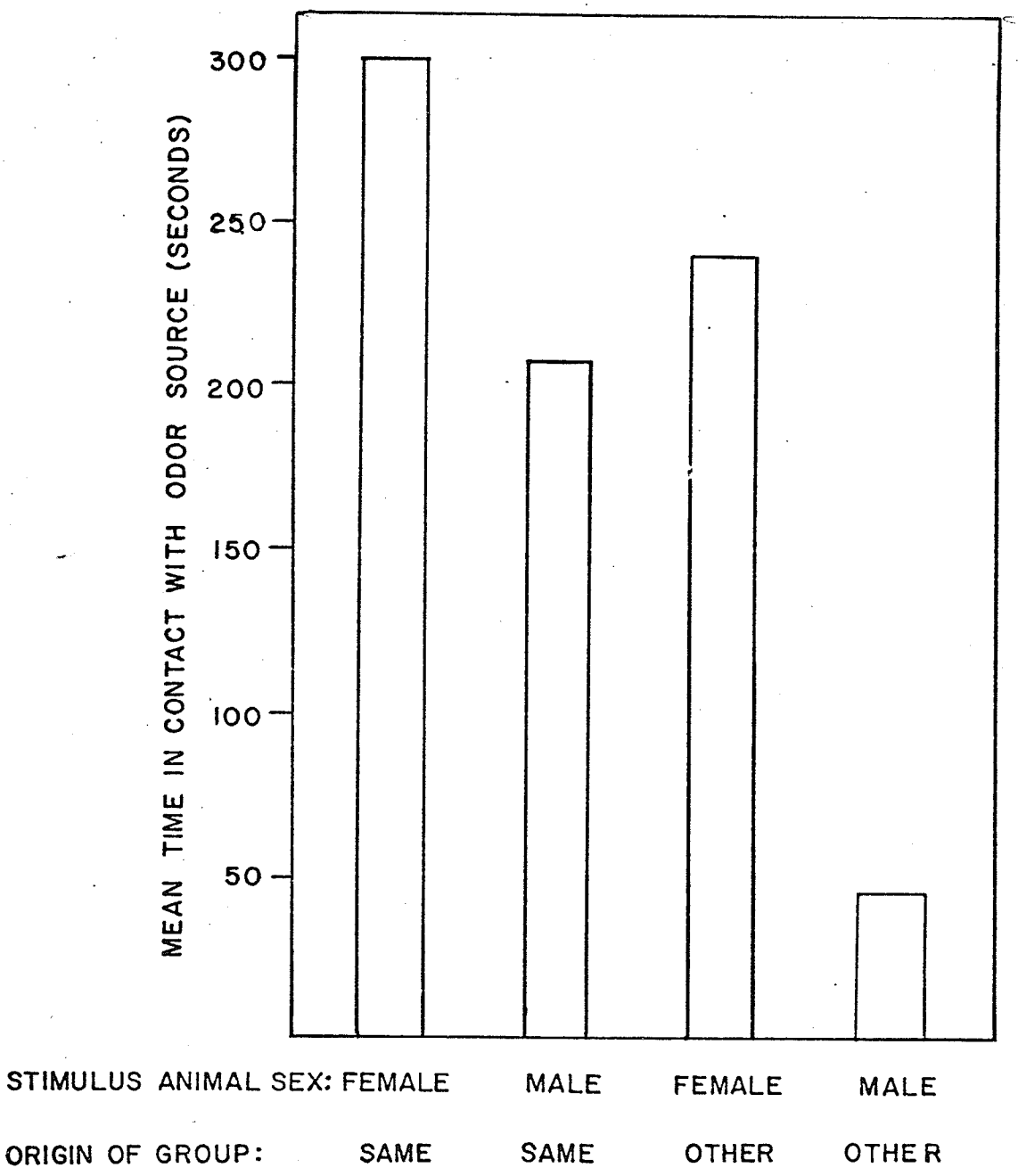
TEST ANIMAL SEX: FEMALE MALE FEMALE MALE
STIMULUS ANIMAL SEX: MALE MALE FEMALE FEMALE


MEAN TIME IN CONTACT WITH ODOR SOURCE (SECONDS)
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100
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STIMULUS ANIMAL SEX: FEMALE MALE FEMALE MALE
ORIGIN OF GROUP: SAME SAME OTHER OTHER

stimulus animals from the same deme. This was true when the comparison was between these odors and those of unfamiliar females ($q = 9.34$, $df = 1/22$, $P < 0.01$) or even male subjects from the test animal's own group ($q = 11.34$, $df = 1/22$, $P < 0.01$). The test subjects allocated both the odors of unfamiliar females ($q = 20.00$, $df = 1/22$, $P < 0.01$) and those of males of their own deme ($q = 18.44$, $df = 1/22$, $P < 0.01$) more contact time than the odors of unfamiliar males. These latter odors, that is, those of unfamiliar males, were contacted for a smaller proportion of the test period than any other origin of group x stimulus animal sex combination.

The analysis of variance for the second dependent variable, the frequency of contact with an odor containing area, is summarized in Table 18. The analysis revealed significant test animal sex x origin of group ($F = 31.71$, $df = 1/22$, $P < 0.001$), origin of group x stimulus animal sex ($F = 44.09$, $df = 1/22$, $P < 0.001$), and test animal sex x origin of group x stimulus animal sex ($F = 61.63$, $df = 1/22$, $P < 0.001$) interactions. The origin of group, stimulus animal sex main effects and the test animal sex x stimulus animal sex interaction did not reach significance at the 0.05 criterion level for this measure.

As before, these significant factors will be looked at in finer detail. Where appropriate, the results of Tukey's post-hoc ratio for mean comparisons (Kirk, 1968) are presented. Unless otherwise specified, only those results significant at the 0.05 criterion level are reported. Table 19 shows the cell means for the various main effects and interactions discussed below.

TABLE 18

Analysis of Variance for Frequency of Contact with an Odor Source

Source	df	MS	F	P
Test Animal Sex	1	3026.25	75.19	< 0.001
Subj. w. Groups	22	40.25		
Origin of Group	1	157.59	3.38	0.080 (NS)
Test Animal Sex X Origin of Group	1	1480.50	31.71	< 0.001
Origin of Group X Subj. w. Groups	22	46.79		
Stimulus Animal Sex	1	68.34	1.78	0.195 (NS)
Test Animal Sex X Stimulus Animal Sex	1	36.26	0.95	0.341 (NS)
Stimulus Animal Sex X Subj. w. Groups	22	38.30		
Origin of Group X Stimulus Animal Sex	1	1449.26	44.09	< 0.001
Test Animal Sex X Origin of Group X Stimulus Animal Sex	1	2025.84	61.63	< 0.001
Origin of Group X Stimulus Animal Sex X Subj. w. Groups	22	32.87		

TABLE 19

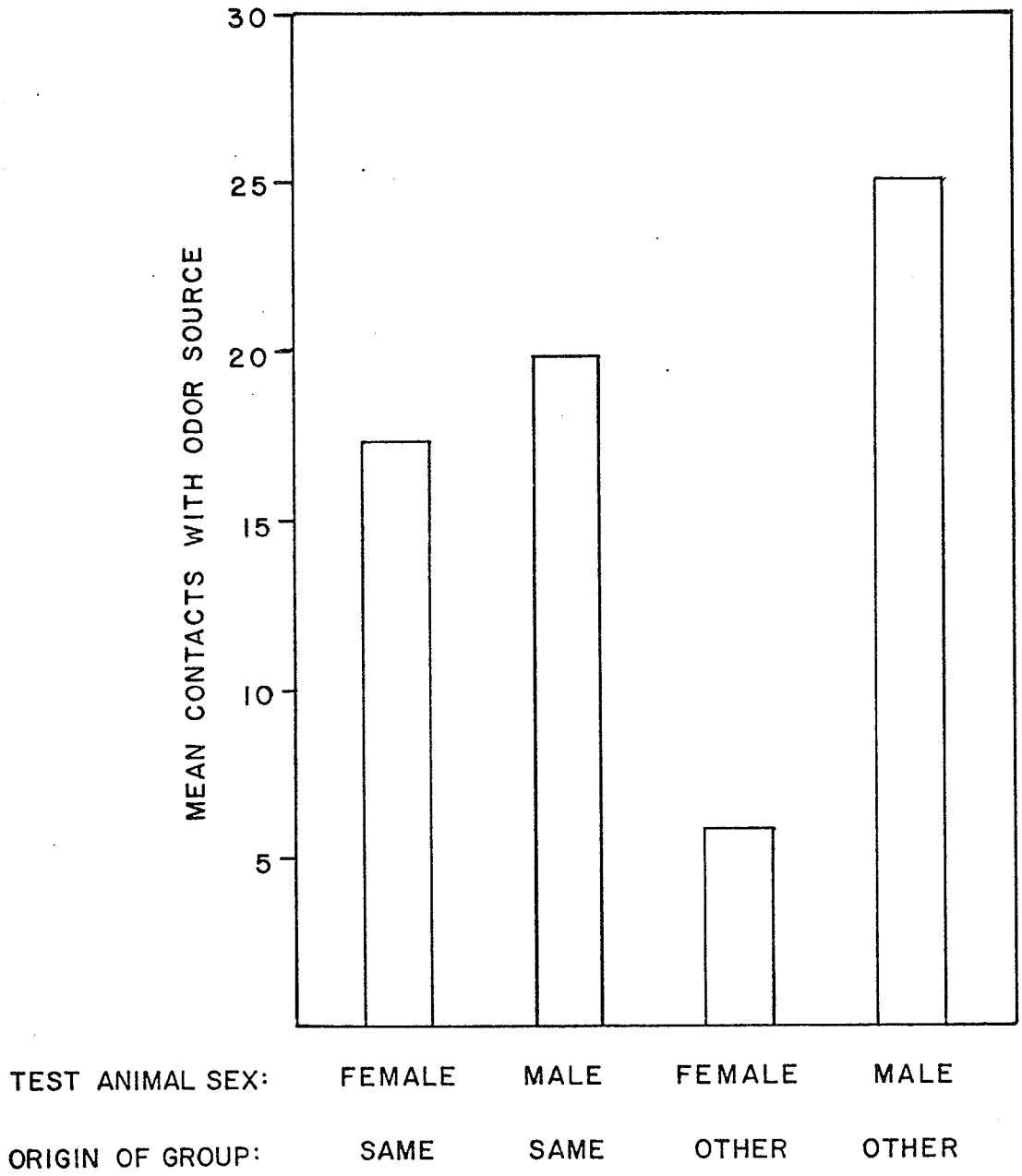
Cell Means for Frequency of Contact with an Odor Source

Origin of Group	Stimulus Odor Stimulus Animal Sex	Test Animal Sex Female	Male	Marginals
Same Group	Male	15.58	11.00	13.29
Same Group	Female	17.08	28.42	22.75
Other Group	Male	3.75	33.25	18.50
Other Group	Female	8.08	16.75	12.42
Marginals		11.13	22.35	16.74

As shown in Table 18, the test animal sex factor was signficant ($F = 75.19$, $df = 1/22$, $P < 0.001$). This finding indicated that males contacted the odor-area more frequently than females did. Although the origin of group factor did not reach significance for this measure, ($F = 3.38$, $df = 1/22$, $P = 0.080$), the interaction of these main effects , the test animal sex x origin of group was significant ($F = 31.71$, $df = 1/22$, $P < 0.01$). Multiple comparisons indicated that while female test animals contacted the odors of members of their own deme more frequently than those of an alien deme ($q = 7.44$, $df = 1/22$, $P < 0.01$), males contacted the foreign odors more frequently than female subjects did ($q = 14.13$, $df = 1/22$, $P < 0.01$). These comparisons are shown in Figure 16.

The major analysis (Table 18) also indicated that the origin of group x stimulus animal sex was significant ($F = 44.09$, $df = 1/22$, $P < 0.001$) even though neither of the component main effects reached significance (origin of group, $F = 3.38$, $df = 1/22$, $P = 0.80$; stimulus animal sex, $F = 1.78$, $df = 1/22$, $P = 0.195$). The relationships which formed this interaction are shown in Figure 17. As can be seen, the odors of female stimulus animals from the same deme as the test subjects' were contacted more frequently by test animals than any other stimulus odor combination. These comparisons were significant for the frequency with which the odors of male stimulus animals from the same deme ($q = 7.75$, $df = 1/22$, $P < 0.01$) or those of females from another group ($q = 8.01$, $df = 1/22$, $P < 0.01$) than the test

subjects were contacted. The odors of males from the other group were not contacted as frequently as females from the same deme as the test subjects. However, their odors were contacted more than those of either males of the same group as the test subject ($q = 4.04$, $df = 1/22$, $P > 0.05$) or females from an adjacent deme ($q = 4.98$, $df = 1/22$, $P > 0.05$). Neither of these last comparisons reached significance. The other comparisons did not approach significance.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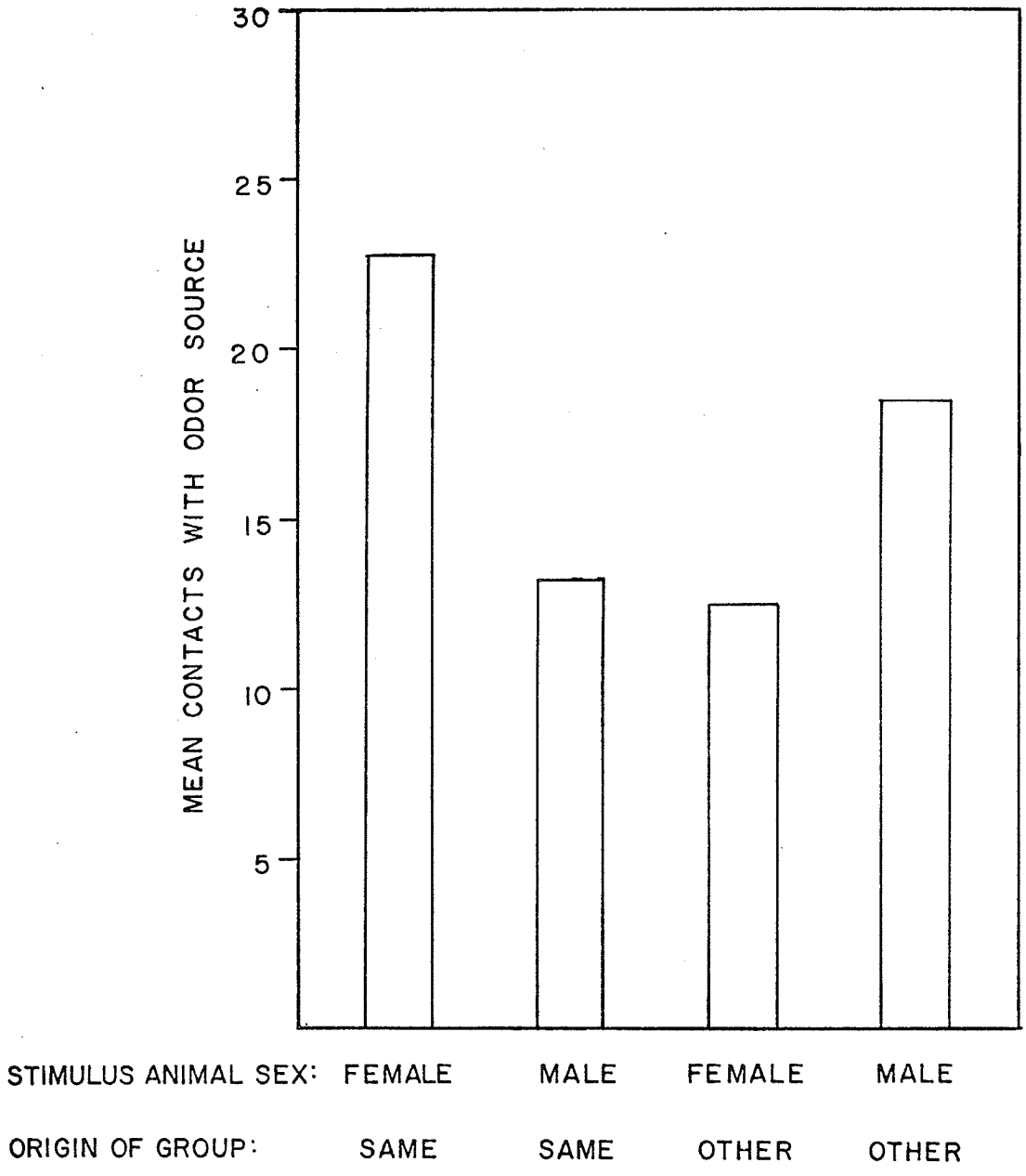
MEAN CONTACTS WITH ODOR SOURCE
TEST ANIMAL SEX:
FEMALE
MALE
FEMALE
MALE
ORIGIN OF GROUP:
SAME
SAME
OTHER
OTHER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MEAN CONTACTS WITH ODOR SOURCE
STIMULUS ANIMAL SEX: FEMALE MALE FEMALE MALE
ORIGIN OF GROUP: SAME SAME OTHER OTHER

EXPERIMENT 6

The results of Experiment 5 essentially mirrored those of Experiment 2. In both experiments subjects spent more time in contact with an area housing the odors of members of their own deme than with that containing another group's odors. Although less time was spent in contact with the odors of alien mice, male subjects contacted these alien odors more frequently than they did those of members of their own deme. The results of all other experiments indicate that these males probably do not prefer the alien odors. Therefore, the same rationale may be utilized to clarify these results as was suggested for similar results reported in Experiment 2. More specifically, it was suggested that the high frequency with the unfamiliar odor may be the result of either the test males's intense investigation of this odor, or marking the alien odor as his own.

The results of Experiment 4 and 5 have indicated that strangeness, rather than previous possible aggressive contact, dictates preferences for the odors of members of the test subjects' own deme and avoidance of alien groups' odors. Therefore, we could ask if the aggression and probable reproductive isolation observed in Experiment 3 would also occur when demes with no previous history of contact were allowed to interact. This was the purpose of Experiment 6.

Method

Subjects

The subjects of Experiment 6 were of the same numbers and composition and were trapped and maintained in the same manner as the subjects for Experiment 3. The major difference between the subjects of this and the previous experiment was that the "other" subjects in this experiment were trapped in areas several miles distant from where the "same" population was captured rather than adjacent to them.

Design

All aspects of the design for Experiment 6 were the same as those described in Experiment 3.

Apparatus

The apparatus for Experiment 6 was the same as that used for Experiment 3.

Procedure

The general procedure used for Experiment 6 was previously described in Experiment 3.

Results

For the same reasons as were given in Experiment 3, the data of Experiment 6 are discussed with reference to central tendencies only. Mean and median aggression frequencies for aggressive encounters between members of the same and unfamiliar groups of

mice are shown in Table 20.

As with the results of Experiment 3, it can be seen that aggression was directed towards members of an unfamiliar deme over twice as frequently as towards members of the same group.

TABLE 20

Mean and Median Number of Aggressive Bouts

Between "Same" and "other" Deme Members

Group	Male	Female	Median	Mean
Same	(N=3)	(N=6)	22	16.67
Other	(N=0)	(N=6)	43	46.33

DISCUSSION

The formation of species, or speciation, can occur in a variety of ways, one of which is the gradual divergence of populations until they become reproductively isolated or non-interbreeding (Mayr, 1963). Reproductive isolation can occur either before mating takes place or after fertilization has occurred. One of the former mechanisms is ethological isolation, which involves the use of signals. These signals are thought to be universally species-specific (Marler, 1957). Several authors have implicated olfactory processes as the signaling mechanism(s) which sexually isolates various species of mice (Bowers & Alexander, 1967; Doty, 1972, 1973; Moore, 1965).

However, evolution takes place within species (Wilcock, 1972) and thus reproductive isolation might be expected to occur below the species level (Wright, 1931). The research reported above has dealt with the question of whether olfactory signals play a role in this isolation.

With respect to the question of the existence of subspecific population groups, house mice (Mus musculus) are often cited as an example of a species whose populations are organized into small, relatively autonomous, reproductively isolated groups (Anderson, 1964, 1967; Anderson & Hill, 1965; Rasmussen, 1964; Reimer & Petras, 1967, 1968; Sealander & Yang, 1969; Sealander, 1970). These demes often occur in areas of sharply defined, patchy habitat discontinuities. Anderson (1964, 1967) reported

that farm buildings, such as granaries, form just such a habitat. The populations used in this study were trapped in farm granaries. They were therefore commensal and trapped in a highly discontinuous habitat. Trapping data from the present experiments indicated that although the granaries were separated by but a few meters resident mice did not cross between the granaries. In fact, although traps were positioned both inside and outside the buildings, no mouse was ever trapped away from its "home" granary. Also, if transient mice were in the study area, none were ever trapped in or near the granaries. The trapping results then indicate that the mice used in this study probably comprised small local populations or demes like those described above. 1

The results of Experiments 1 and 2 suggest the involvement of olfactory variables in the reproductive isolation of demes. In Experiment 1, both male and female subjects spent more time near air-transported odors originating from the same deme as themselves than near the odors of adjacent deme members. Eisenberg and Kleiman (1972) speculated that this differentiation would be likely.

Schultz and Tapp (1973) argued that preferences for the odors of same group members may facilitate group cohesion. This process probably involves the recognition of familiar group or individual odors. The findings of Bowers and Alexander (1967), Hahn and Simmel (1968) and Husted and McKenna (1966) indicate that this discrimination is possible. Lockley (1961) reported that rabbits frequently mark one another in encounters between colony members. He argues that such marking could be a mechanism

for reinforcing familiarity between group members.

However, group cohesion and subsequent reproductive isolation from adjacent demes is more easily achieved if the members of other groups are also avoided. Both male and female subjects in Experiment 1 avoided the odors of mice trapped nearby, who were members of another deme. That is, they spent more time near a fresh air source than near the odors of "other" deme members. Female subjects spent more of the test period avoiding these "other" odors than males did. These findings are consistent with the finding that strange mice are not readily integrated into established populations (Anderson, 1964; Crowcroft & Rowe, 1963; Lidicker, 1976; Murray, 1967; Reimer & Petras, 1967; Wolfe & Summerlin, 1968).

Several studies (Bowers & Alexander, 1967; Dag & Windsor, 1971) have shown that the sex of conspecifics can be identified by odor cues. The results of Experiment 1 indicated that the subjects of this experiment distributed their preferences for air-transported odor stimuli by the sex of the stimulus animal. Basically, both male and female test subjects preferred the odors of female stimulus animals over those of males. Schultz and Tapp (1973) report that although the results of many tests are conflicting, generally the data indicate that this pattern of preferences is to be expected. Conversely, the odors of males were avoided by test subjects of both sexes. That is, more time was spent near the fresh air source than near the odors of males. The avoidance of male odors by other males is fairly well documented (Doty, 1973; Moore, 1965). However, little actual

support can be cited for the finding indicating that females also avoid these odors.

Experiment 1 also indicated that the odors of females from the same deme as the test subject were most preferred. This preference was followed in descending order by the time spent in the vicinity of odors originating with females of an adjacent deme, males from the same deme and finally, males from the adjacent deme. Not only was this last odor least preferred, but it was actually avoided. These findings reflect both the preferences for female stimulus odors and the odors of the same deme members previously discussed. It is interesting that the odors of females from an adjacent deme were preferred over those of males of the same deme as the test subject. Since a discussion of reproductive isolation makes sense only if females of reproductive condition are considered, all female subjects used in these experiments were in estrous. These results may have been different had diestrous females been included in the tests.

Although evidence shows that even alien females are not readily accepted into established demes, (Anderson, 1964, 1967; Lidicker, 1976; Reimer & Petras, 1967; Wolfe & Summerlin, 1968) their acceptance would probably not constitute much of a threat to the groups' cohesion within the limits imposed by available food supply and space, for females are less aggressive than males (Lagerspetz, 1964). Diestrous females would probably threaten group cohesion least. In addition, the acceptance of non-threatening females into an established group would serve to

broaden the gene pool and enhance the inclusive fitness of the male members of the deme. That is, the net genetic representation of those males in the genetic composition of succeeding generations would increase (Barash, 1977). Since males are more aggressive and have the most to lose in fitness, so to speak, by allowing other males to breed with females from their group, it would not be suprising to find that the odors of males from an adjacent deme were avoided since these animals pose the greatest threat to both group cohesiveness and inclusive fitness.

These findings support both the data-based and theoretical conceptions of rodent society advanced here and in other authors' works (Anderson & Hill, 1965; Brown, 1953; Crowcroft & Rowe, 1963; Lidicker, 1976; Murray, 1967; Reimer & Petras, 1967). Further, Ralls (1971) stated that many animals scent mark more frequently than usual following an encounter with an individual with whom they normally do not associate. She contended that in a variety of species the scents of conspecifics produce similar effects and cited reports of sugar gliders, rabbits, hamsters and marmosets as examples of animals which mark more frequently than usual when exposed to the odors of members of another social group or to strangers.

In fact, olfactory variables have been implicated in rodent aggression (Archer, 1968; Lee, 1970; Mugford & Nowell, 1970, Roparz, 1968) and dominance (Carr, Martorano & Krames, 1970; Desjardins, Maruniak & Bronson, 1973). Likewise, olfactory-mediated behaviors may be the basis for, or at least reinforce, territorial maintenance (Nyby et al., 1970; Theissen & Dawber,

1972). Territorial maintenance is believed involved in the formation of and segregation of intraspecific population units (Anderson & Hill, 1965; Theissen & Dawber, 1972; Reimer & Petras, 1967, 1968; Sealander, 1970).

Therefore, olfactory variables associated with territorial marking may be important in isolating small groups, such as demes, within the general population. The results of Experiment 1 indicated that this may be the case. However, that experiment utilized the air-transported odors an individual "carried" around with him. Presumably, these would be sebaceous in origin. Experiment 2 attempted to assess preferences for and frequency of investigation of odors which are presumably more directly associated with the territorial marking (e.g. urine and feces) than the odor stimuli used in Experiment 1. These odorous products have often been associated with territorial border markings (Anderson & Hill, 1965; Crowcroft, 1955; Crowcroft & Rowe, 1963; Eisenberg, 1962; Shillito, 1963). These marking posts presumably communicate "ownership" of the area they enclose (Eisenberg, 1962; Eisenberg & Kleiman, 1972; Ralls, 1971). Marking posts commonly elicit intense investigation by animals new to the area (Eisenberg, 1962, 1963; Eisenberg & Kleinman, 1972) or avoidance by individuals who have previously been defeated by the territory "owner" (Thiessen & Dawber, 1972). If territorial marking effectively delimits the movement of, and integration of small, local populations, like demes, reactions to urine and feces markers should depend upon whether the "intruder" is a member of the marking animals' deme or another, adjacent deme. The results

of Experiment 2 indicate that these differences do occur.

Both male and female subjects spent more time in contact with an area containing the excretory products of members of their own deme than one containing the products of another adjacent deme. This result was similar to the findings of Experiment 1 and indicates that territorial markings, as well as body odors, can influence the preferences of mice for the odors of animals of either the same, or another group than themselves. In other words, mice approaching a marked area will avoid it if it has been marked by the members of an adjacent deme. The present finding reflects and in part clarifies the trapping results which indicated a non-existent cross-over rate between the areas occupied by adjacent demes. As has been mentioned, the trapping results of this study coincide with those reported by other authors (Anderson, 1964, 1967; Reimer & Petras, 1968; Crowcroft and Rowe, 1963, Lidicker, 1976; Sealander 1970). Also, in keeping with the results of Experiment 1, both male and female subjects preferred or spent more time in contact with the odorous products of female stimulus animals than with those of males. Again, we can assume that since females are less aggressive than males (Lagerspetz, 1964), the areas marked by them would probably constitute a lesser threat than the male-marked areas. This explanation may also account for the manner in which subjects distributed the time they were in contact with a male or female odor source originating from either their own, or an adjacent deme. Relatively little time was spent contacting the odor area of an adjacent group male as compared to either that of a male

from the same deme as the test subject or a female from either deme.

Contact with an alien group male could conceivably pose a threat to both the cohesiveness of the invaded group, and its physical well being. Odorous deposits which indicate that contact with such an individual is likely would best be avoided. The results of both Experiments 1 and 2 indicate that this avoidance does occur.

The contact frequency data of Experiment 2 indicate that males contacted an odor source more often than females did. Since males are more aggressive than females (Lagerspetz, 1964) and scent mark and patrol territorial boundaries more frequently than females (Eisenberg, 1963; Eisenberg & Kleiman, 1972; Johnson, 1973; Ralls, 1971; Shillito, 1963), their higher rate of odor investigation would be expected. In addition, we might speculate that since males would have the most to lose by the disruption of the established group of which they are members, inclusive fitness could be enhanced and protected by investigating a strange odor. Males would then investigate the odors of intruding males frequently, but not spend much time near the potentially dangerous odor source. The results of Experiment 2 indicate that males behaved in this way. This explanation then, may in part account for the seemingly contradictory evidence indicating that while the odors of males from the other group were investigated, or contacted more frequently than those of males from the same group, subjects spent more time in contact with male members of their own group rather than with the males of

other demes. In addition, the odors of females from the same deme as the test subject were contacted longer and more frequently than any stimulus animal sex x origin of group odor combination. There are several possible explanations for this result. First, same group females may constitute the only totally non-threatening odor source. Or, because the group's cohesion and fitness are threatened when member females are left unattended, these odors may be contacted longer and more frequently to insure the safety of their owner against violation.

Finally, we can ask how these findings would be reflected in a situation in which a male was given access to females of his own and an adjacent deme. Experiment 3 was designed to answer these questions. Males could enhance their fitness by breeding or attempting to breed with the adjacent group females first. If they adopted this policy, their genes would become more frequent in the overall gene pool. This would be reflected in a higher frequency of insemination (as indicated by the presence of vaginal plugs) of the adjacent deme females. Also, if these females were imseminated by a strange male pregnancy blockage would probably not cancel out his efforts if the female were to return to her own deme (Bruce, 1960). The blockage of pregnancy occurs only when the female is not familiar with the non-stud male's odors. In this case the "non-stud" male would be a member of her own deme.

On the other hand, males of a female's home deme would probably share more genes in common with her (Anderson, 1964, 1967; Petras, 1967; Reimer & Petras, 1968; Sealander, 1970;

Sealander & Yang, 1969). Therefore, a female would enhance her fitness most by breeding with males from her own group. Support for this prediction is found in reports indicating that females often aggressively defend a group's home territory against invasion by strange mice (Anderson & Hill, 1967; Crowcroft & Rowe, 1963; Eibl-Eibesfeldt, 1950, 1970). The results of Experiments 1 and 2 indicate that females spend less time near the odors of males from an adjacent deme than they do near the odors of males from their own deme. This suggests that they may not allow adjacent deme males to breed with them.

When several (2 in each replication) females were caged with like numbers of females and a male from an adjacent deme, aggressive exchanges were common. As has been mentioned, the planned measure, the incidence of vaginal plugs in same and other deme females, was abandoned in favor of a frequency of aggressive bouts measure. Observations indicated that females were aggressive towards the members of the adjacent deme whenever contact occurred. This finding indicates that in a more natural situation a female could, by rejecting the advances of an adjacent deme male, or perhaps escaping, or avoiding him altogether, assure reproductive isolation and protect the continuity of the local gene pool of which she was a member.

The finding that aggression also occurred between members of the same deme is more difficult to explain. For instance, Wolfe and Summerlin (1968) reported that aggression was rare among animals trapped near one another. Also, Crowcroft and Rowe (1963) found that aggression was rare in family groups. Perhaps

this within-deme aggression was promoted because both "same" and "other" deme members were in the apparatus at the same time.

So far then, this experimental series has shown that small local populations of house mice trapped in adjacent granaries prefer the odors of members of their own deme and avoid those of neighboring deme members whether the odors are air-transported body odors or originate from urine and feces which could represent territorial markers. The odors of adjacent deme males were actively avoided. However, this odor was also investigated most frequently by the male subjects, the defenders of the deme's territory. These results lend support to the notion that reproductive isolation between these autonomous local populations was at least mediated or reinforced by the production and detection of odorous substances associated with the aggressive defense of territorial limits. The results of Experiment 3 indicated that these hypotheses concerning olfactory based reproductive isolation were reflected in the females' aggressive rejection of suitors from neighboring demes. Such female aggression may be the basis for reproductive isolation.

This research was based, at least in part, upon statements by Thiessen and Dawber (1972) indicating that the odors mediating reproductive isolation between local populations are reinforced by, or perhaps dependent upon, aggressive encounters between members of the neighboring demes. However, several studies have reported that avoidance of an odor, or aggression toward its carrier, are positively correlated with the degree of strangeness of the odor (Archer, 1968; Mugford & Nowell, 1970; Roparz, 1968).

Therefore, reactions discussed thus far may not depend upon previous aggressive encounters with neighbors as Thiessen and Dawber (1972) have speculated.

The second experimental series was designed in part to clarify this problem. It is likely that members of neighboring demes would have had some previous contact, even if it consisted only of a familiarity with their neighbors' odors rather than aggressive confrontation. However, it is extremely improbable that the members of demes whose home ranges were separated by several miles would have had any previous contact with one another. This experimental series therefore attempted to test the proposition that the potential for reproductive isolation is not dependent upon prior contact or familiarity.

The results of the second experimental series (Experiments 4, 5, and 6) essentially duplicated those of the first set of experiments (Experiments 1, 2, and 3). There were some minor exceptions to this generality. For instance, in Experiment 4, female subjects avoided male odors more than male subjects did. Just the opposite result was found for Experiment 1. Also, in Experiment 1, more time was spent with the odors of adjacent group females than with males of the same deme as the test subject. Again, this relationship was reversed in Experiment 4. Although these inconsistencies disrupt the continuity between the results of the first and second experimental series, they probably do not affect the generalized discussion which follows.

The general resemblance of results for the first and second experimental series indicates that prior experience with an odor

complex is not necessary for reproductive isolation to occur. Evidently, a deme member need only be familiar with the odors of his own deme to achieve this potential. This strategy may economize the effort a mouse must expend in achieving reproductive isolation and subsequent group social cohesiveness. That is, an animal need not learn or recognize a large number of periodically changing odor complexes and the appropriate reactions to each. Instead, only the odor(s) of the deme to which it belongs require recognition. All other colony odors evoke either avoidance or aggression. This process, then, can assure group autonomy and cohesiveness which allow exploitation of the environment without destructive overcrowding and yet provide the potential for acceptance into another group upon dissolution of the original group of membership.

Although the results reported here do not show that the above speculations are valid, such could be the case. It appears that the mutual avoidance of strangers and their odors is a part of territorial exclusion as it is commonly conceived. We can therefore ask why a territorial system based on such costly behaviors as marking, patrolling, aggression and dominance is maintained over a system based solely upon the avoidance of non-deme odors. Brown (1964) has argued that a territorial system benefits territory owners by assuring access to certain limited resources, such as mates, food supply and shelter. Brown (1964) has also pointed out that territorial possession enhances the inclusive fitness of male territory owners. Territorial boundaries allow owner-males exclusive access to the females who

are residents of the territory. The potential of reproducing with these females is limited for males who are not members of the deme. Therefore, territorial ownership can be a means whereby a male can increase his contribution to the gene pool over non-territory holders.

Also, the attachment to, or preference for, other members of the deme by a group member may help to maintain the cohesiveness required for resource protection. In addition, if both males and females avoid any non-deme mouse odors, this group cohesion is enhanced. The avoidance of non-deme members may also provide an economical means of assuring exclusive use of resources and stable social relationships within a territorial system. Marking, patrolling and other behaviors associated with odor-marked territorial holding, although costly, may be more economical in terms of social cohesiveness, resource allocation and protection than a system which dictates the avoidance of all other mice. Therefore, olfactory-mediated mutual avoidance may contribute to the maintenance of a territorial system.

Territorial social systems may also restrain a population's reproductive potential, thereby helping to prevent the over exploitation of available resources (Wynn-Edwards, 1962). Davis (1958) presented evidence indicating that the social structure of house mouse populations was territorial at low population levels only. As the number of mice increased, more and more social disintegration occurred until at high densities the territorial type social structure was replaced by one of interpersonal aggression and mutual avoidance.

The importance of this research centers around the finding which indicates that odor production and detection play an important role in the reproductive isolation of small local populations. Also, this research indicated that prior experience with a neighboring group or its odors is not required for the potential of ethological isolation between demes to be a reality. This research points to the possibility of group odor complexes playing an important role in rodent population distribution, regulation and structure.

In addition, this research opens the door for a plethora of related future research. First, the apparatus used for Experiments 1 and 4 could be modified to record not only the time spent in the vicinity of an odor source, but also the frequency of entry into an odor compartment. This information would indicate whether the test subject investigates strange body odors frequently but spends little time near them. Such reactions were hinted at by the results of Experiments 2 and 4. Also, the results of Experiments 3 and 6 could be clarified by offering potential breeding situations between "same" and "other" groups other than those described here. For instance, a male could be allowed access to "other" group females without females of his own group being present. The potential for aggressive reactions in this situation is now known and other, more inclusive dependent measures could be planned. An experiment of this type may help determine if reproductive isolation <u>actually</u> occurs between demes.

In addition, since only estrous females were used in the above

experiments, it might be informative to conduct a similar series of experiments utilizing diestrous female test and stimulus animals. The results of this experiment would be important, for diestrous females may constitute the least threat to an established deme's cohesion. These females may occasionally gain entrance to a deme other than their own. They may then represent an important source of gene flow within the gene pool as a whole.

In Experiment 1 it was mentioned that in the particular year the subjects for these experiments were sampled, population density was quite low. The distribution of demes within granaries and the numbers of animals comprising a deme may also have been unusual. These factors may have placed limitations upon the experiments described. Therefore, it may be beneficial to reevaluate the population dynamics in another time or another place. Also, if the distribution and density of the demes sampled were found to be unusual, the results discussed here should perhaps also be reevaluated.

Thus far, a great deal of emphasis has been placed upon olfactory reproductive isolation between groups. However, statements to this effect were not intended to imply that genetic interchange between demes does not occur. If no genetic exchange between demes took place, Mus musculus's identity as a separate species would long ago have disappeared. Given a demetic type social system within which reproductive isolation occurs between the small local populations, how is the species' genetic integrity maintained? Anderson (1967) speculated that gene flow is promoted by the occasional acceptance of a transient female into

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APPENDICES: A AND B

APPENDIX A: Summary of Analysis of Variance Including Replications for Experiments 1, 2, 4, and 5.

EXPERIMENTS 1 AND 4

Source	Odor, E1	Fresh Air, E1	Odor, E4	Fresh Air, E4
Replications	0.393	0.827	0.360	0.222
Test Animal Sex	0.068	0.122	<0.001*	0.830
Replications X Test Animal Sex	0.104	0.415	0.556	0.964
Origin of Group	<0.001*	<0.001*	<0.001*	<0.001*
Origin of Group X Replications	0.970	0.162	0.937	0.267
Origin of Group X Test Animal Sex	0.045*	0.017*	<0.001*	0.863
Origin of Group X Replications X Test Animal Sex	0.633	0.695	0.173	0.627
Stimulus Animal Sex	<0.001*	<0.001*	<0.001*	<0.001*
Stimulus Animal Sex X Replications	0.968	0.823	0.295	0.071
Stimulus Animal Sex X Test Animal Sex	0.604	0.526	0.008*	0.346
Stimulus Animal Sex X Replications X Test Animal Sex	0.490	0.650	0.248	0.919
Origin of Group X Stimulus Animal Sex	0.075	<0.001*	0.195	<0.001*
Origin of Group X Stimulus Animal Sex X Replications	0.605	0.015*	0.008*	0.371
Origin of Group X Stimulus Animal Sex X Test Animal Sex	0.163	0.001*	<0.001*	0.574
Origin of Group X Stimulus Animal Sex X Replications X Test Animal Sex	0.797	0.528	0.444	0.369

Continued...

APPENDIX A - Continued.

EXPERIMENTS 2 AND 5

Source	Time Ex.2	Contact Ex.2	Time Ex.5	Contact Ex.5
Replications	0.099	0.096	0.407	0.451
Test Animal Sex	0.985	<0.001*	0.122	<0.001*
Replications X Test Animal Sex	0.689	0.799	0.687	0.044*
Origin of Group	<0.001*	0.408	<0.000*	0.089
Origin of Group X Replications	0.318	0.868	0.296	0.599
Origin of Group X Test Animal Sex	<0.001*	<0.000*	0.001*	<0.001*
Origin of Group X Replications X Test Animal Sex	0.745	0.269	0.826	0.385
Stimulus Animal Sex	<0.001*	0.503	<0.001*	0.155
Stimulus Animal Sex X Replications	0.427	0.656	0.542	0.049*
Stimulus Animal Sex X Test Animal Sex	0.006*	0.133	<0.001*	0.294
Stimulus Animal Sex X Replications X Test Animal Sex	0.629	0.741	0.852	0.394
Origin of Group X Stimulus Animal Sex	0.008*	<0.001*	0.001*	<0.001*
Origin of Group X Stimulus Animal Sex X Replications	0.740	0.524	0.942	0.709
Group of Origin X Stimulus Animal Sex X Test Animal Sex	0.976	<0.001*	0.267	<0.001*
Group of Origin X Stimulus Animal Sex X Replications X Test Animal Sex	0.736	0.033*	0.710	0.110

*Significant results.

APPENDIX B : Original Design and Procedure for Experiment 3.

Design

A 2 x 2 chi-square with one degree of freedom was used for the overall analysis. The independent variable, the population of the female, was described as either the "same" as that of the male subject, or "other" than that of the male. The dependent variable was the presence or absence of a vaginal plug. Since the two groups ("same" and "other") are independent and the data are in terms of frequencies in discrete categories, the chi-square test of independence is the appropriate statistical test.

The total numbers of vaginal plugs for each "same" or "other" population was summed over three replications of the experiment and the chi-square analysis performed on these frequencies. No subject, male or female, was used in more than one test. However, all subjects used in Experiment 3 had previously been subjects in Experiment 1 and 2 for the reasons outlined above.

Procedure

To assure that the female subjects of Experiment 3 were in a sexually receptive condition, they received the same series of hormone injections as has been described for the other experiments. Vaginal smears were taken from female subjects on the day of testing to verify their estrous state. One hour after the last vaginal smear was taken the estrous female subjects from each population were randomly assigned to a test compartment of the four compartment chamber. A test male was then placed in

another compartment and the trial begun. The test animals were allowed to interact freely within the apparatus for two hours. All subjects were then removed and returned to their respective home cages. Seventy-two hours after the subjects were removed frcm the test apparatus all females were examined for the presence of vaginal plugs, an indication of insemination.

Three replications of this experiment were conducted. Each replication utilized new subjects. The apparatus was thoroughly cleaned with a strong laboratory cleanser (Alconox) and water, and the sawdust floor renewed before each replication.