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

By

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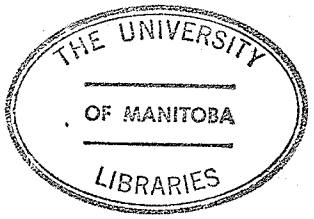

ABSTRACT

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.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICIES

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-