LABORATORY STUDIES OF ESTRUS INDUCTION AND PREGNANCY MAINTENANCE IN VOLES (MICROTUS)

By

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To my parents,
John D. and Phyllis A. Taylor

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TABLE OF CONTENTS

APPENDICES

Abstract of Thesis Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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Studies were conducted to investigate the role of male-produced chemosignals in successful reproduction of female Microtus.

In the first study species differences in male-induced estrus were examined in nulliparous and parous montane (M. montanus), meadow (M. pennsylvanicus), and prairie voles (M. ochrogaster). Nulliparous prairie voles required a greater duration of male exposure for receptivity than nulliparous montane voles. Nulliparous meadow voles evidenced an extremely low copulation rate. Parous prairie voles required a greater duration of male exposure for receptivity than parous montane and meadow voles. Parous montane and meadow voles did not differ in duration of male exposure needed for receptivity. Only montane and meadow voles were observed to copulate without prior direct exposure to males.

Parous females had higher copulation rates than nulliparous females. When isolated from males, prairie voles had more leukocytes and fewer cornified cells in vaginal smears than montane or meadow voles. Vaginal smears were only marginally predictive of behavioral receptivity.

Stud-male mediated maintenance of pregnancy in montane voles was investigated in subsequent studies. Stud presence after copulation increased the likelihood that nulliparous, but not parous, females carried a litter to term. Male post-copulatory presence effected through "visits" appeared to increase the likelihood of a pregnancy being maintained proportional to the amount of post-copulatory male exposure. Neither the opportunity for extended duration of copulation nor limited mating with an equal period of extended male presence enhanced pregnancy in nulliparous females. Male presence beyond the initial 24 hours after the onset of copulation is required to enhance pregnancy success.

These results are discussed in terms of some general issues in the study of primer pheromones. House mice (Mus) and voles (Microtus) are compared regarding primer responsiveness. The role of primers in reproductive strategies of voles is discussed. Finally, species differences among Microtus in primer responsiveness are discussed in relation to species differences in social organization.

CHAPTER 1
GENERAL INTRODUCTION

Priming Pheromones and Mammalian Reproduction

Since the original observations of van der Lee and Boot (1955), Whitten (1956), and Bruce (1959), it has become apparent that chemosignals can have a remarkable influence on mammalian reproductive physiology. These chemosignals, known as priming pheromones, influence reproduction by stimulating a change in endocrine or neuroendocrine activity. The role of priming pheromones in mammalian reproduction, in terms of both proximate mechanisms and ultimate (evolutionary) explanations, is a topic of considerable research activity. Known or suspected primers occur both within and between both sexes (see Brown, 1985).

Some of the best known primer effects include the influences of male-released chemosignals on the reproductive physiology of females. House mice (Mus musculus) have been most commonly studied. The odors of males have been shown to influence females at three main junctures: puberty, estrus (sexual receptivity), and pregnancy.

Vandenbergh (1967) first observed that contact with adult males hastened the onset of puberty in female house mice. The pheromone is present in the urine of males and dependent on adequate circulating levels of testosterone

(see Vandenbergh, 1983). Chemical and tactile cues combine to produce the maximal effect (Bronson & Maruniak, 1975; Drickamer, 1974). Puberty acceleration is thought to influence reproductive success by decreasing the age at which females may first breed successfully (Bronson, 1979a,b; Drickamer, 1986).

In adult female house mice the odors of males advance ovulation and hasten the onset of sexual receptivity. Female mice have shorter estrous cycles when housed with males than when housed alone, or in all female groups (Whitten, 1956, 1959). Ovulation advancement is thought to influence reproductive success by optimizing the time of ovulation (see Bronson, 1979a,b). At times when nutritional and other requirements are favorable, and both a male and female are present, breeding can occur very rapidly.

Chemosignals from males can also block the implantation of fertilized ova in the uterine wall, resulting in a pregnancy block commonly known as the Bruce effect (Bruce, 1959). In house mice this pregnancy block occurs when a female, after mating with a stud male, is exposed to a strange male during a critical period before implantation (see Brown, 1985). The Bruce effect has been the subject of much adaptive theorizing; proposed benefits may accrue to the female or blocking male (e.g., Dawkins, 1976; Labov, 1981; Schwagmeyer, 1979; Storey, 1986a). Bronson and Coquelin (1980) have argued that such elaborate adaptive theorizing is largely unwarranted, as the conditions

necessary for the Bruce effect are unlikely to occur in the field.

Bronson (see Bronson, 1979a,b; Bronson & Coquelin, 1980; Bronson, 1989) has reviewed and synthesized the immense house mouse literature and developed an elaborate "house mouse model" of the pheromonal cueing of reproduction. Key features of the model include mutual stimulation between adult males and adult or prepubertal females, and female-female antagonism of the male priming action on the physiology of prepubertal females. He views the pheromonal cueing system of house mice as an important component of an adaptive complex of traits allowing ecological opportunism and extraordinary success in global colonization.

Voles and Priming Pheromones

While much research has been conducted with house mice, there are a number of basic reasons that make voles of the genus Microtus excellent subjects for the investigation of primer actions. First, most vole species can be easily maintained and adapt fairly well to the laboratory. The nature of the procedures and the requirements of experimental control largely confine the study of basic primer actions to the laboratory. Second, there is a well developed literature on the biology of Microtus (see Tamarin, 1985), including behavior both in the laboratory and in the field. This allows laboratory investigation of priming effects to proceed in light of knowledge gained

regarding the behavior of animals in the field. There is very little information on the field behavior of some common laboratory rodents (e.g., Mongolian gerbils, Siberian hamsters). Finally, voles are likely to be quite dependent on a pheromonal cueing system. Bronson (1979b) has noted they possess a number of characteristics associated with pheromonal cueing of reproduction including 1) a capacity for explosive reproduction, 2) an existence at times in over-dispersed and temporally unstable populations, and 3) being ecologically opportunistic.

In addition to the basic reasons described above, the study of olfactory influences on reproduction in Microtus has potential for increasing the scope and generality of our knowledge about the actions of priming pheromones. While classic primer effects such as puberty acceleration (e.g., Hasler & Nalbandov, 1974; Sawrey, 1989) and the Bruce effect (e.g., Stehn & Jannett, 1981) have been demonstrated in voles, it is clear that the manifestation of primer action in Microtus can differ considerably from house mice. First, adult Microtus females do not show a spontaneous estrous cycle and are dependent to varying degrees on contact with male chemosignals for the induction of estrus (see Richmond & Stehn, 1976; Sawrey & Dewsbury, 1985). Also unlike house mice, the temporal "window" during which the Bruce effect can occur appears to extend beyond implantation in voles (Stehn & Richmond, 1975; Storey, 1986b). There is also evidence that male chemosignals present after copulation may

increase the probability that a pregnancy will be maintained (Berger & Negus, 1982; Brown, 1985; Richmond & Stehn, 1976). This effect has not been reported in house mice.

Furthermore, unlike house mice, the use of voles as subjects provides an excellent opportunity for species-level comparative research into the action and function of primer pheromones. Much of the current interest in Microtus stems from work indicating contrasting patterns of social organization among different species (see Wolff, 1985). These differences in behavior should, at least in part, result from stable species differences in the behavioral tendencies of individuals that make up the species. When studied in the laboratory, species-typical characteristics of physiology and behavior emerge (see Table 1) that may drive the contrasting patterns of social organization in the field. While all Microtus species are likely to be quite responsive and dependent on priming pheromones, subtle species differences in the utilization and responsiveness to primers may, like the characteristics listed in Table 1, contribute to observed species differences in social organization and mating systems.

Plan of the Dissertation

The studies reported are focused on the influence of male chemosignals on reproductive function of females in relation to estrus (vaginal estrus and behavioral receptivity) and pregnancy. Three major considerations permeate this work. First, studies were designed to

contribute to the knowledge of primer functions in Microtus. Pheromonal cueing of reproduction in voles (Microtus) has received much less study than pheromonal cueing in house mice (Mus). The present understanding of the primer role in the reproductive strategies of house mice would clearly not be possible without the existing comprehensive data base. Second, it is recognized that the primer actions studied herein are related in terms of stimuli and mechanism as part of a larger pheromonal cueing system. This cueing system involves direct links to the brain-pituitary-gonadal axis and thus allows the tailoring of reproductive efforts to the prevailing environmental conditions (Bronson, 1979a). In the early stages of study, mammalian primers were often viewed as distinct "effects", each named for their discoverers (e.g., Whitten effect, Bruce effect, Vandenbergh effect, etc.). Viewing each distinctly hindered an understanding of an overall priming system and the role of priming pheromones in reproductive strategies. Third, the advantages of species level comparative analysis possible with Microtus are recognized and utilized. This perspective may allow insights into the function of primers in natural populations that are just not possible with single species research.

An investigation of male-induced estrus in montane voles (M. montanus), meadow voles (M. pennsylvanicus), and prairie voles (M. ochrogaster) is reported in Chapter 3. This study was undertaken to provide more comparative

information on the influence of male exposure on vaginal cytology and behavioral estrus in these species. Species differences in female responsiveness to estrus-inducing chemosignals that may contribute to differences in social organization and mating systems were investigated. The relationship between changes in vaginal cytology and behavioral estrus was also explored. The background and rationale for this study are developed more fully in Chapter 3.

An investigation of stud-male mediated maintenance of pregnancy in montane voles is reported in Chapter 4. Stud-male mediated maintenance of pregnancy refers to the increased likelihood that a female will carry a pregnancy to term if she remains exposed to the stud after copulation. The study of this effect is of interest for at least two reasons. First, pregnancy maintenance is a little known primer effect that has not been reported for Mus, but has been reported in other genera, and may have important implications for behavior in the field. Second, since it involves post-copulatory interaction between the female and the stud male, its manifestation is likely to vary with social organization and mating systems. The pregnancy maintenance phenomenon was studied in detail in montane voles. The experimental manipulations are relevant to the behavior of montane voles and how they may differ from other vole species regarding social organization and mating systems and this primer effect. The background and

rationale for the study of pregnancy maintenance are developed more extensively in Chapter 4.

In the final chapter the experimental results of this dissertation are placed in a larger perspective of some general issues in the study of primer pheromones and mammalian reproduction. House mice (Mus) and voles (Microtus) are compared with respect to primer responsiveness. The role of primer pheromones in the reproductive strategies of voles is discussed. Finally, species differences among Microtus in primer responsiveness are discussed.

Biology of Montane, Meadow, and Prairie voles

Voles of the genus Microtus are small (35 - 60 g) robust animals with squat bodies, blunt muzzles, small eyes and ears, and short legs and tails. The pelage generally consists of some shade of brown, ranging from light brown to very dark. Some species have a grayish tint. Carleton (1985) provides an extensive discussion of the macroanatomy of Microtus.

Microtus generally inhabit a grassland environment that provides cover for their activities. Most species feed extensively on grasses and sedges; seeds, roots, dead vegetation, and insects may be eaten when green vegetation is not available (Getz, 1985). Mesic or wet habitats are inhabited by most species (Getz, 1985). Montane voles are found in the grass and sedge dominated mountain valleys of the northwestern United States (Getz, 1985; Jannett, 1980).

Meadow voles are found throughout the northern United States and Canada (Hoffmann & Koeppl, 1985). Prairie voles inhabit the grassland habitats of the central United States (Getz, 1978, 1985). The original habitats of prairie and meadow voles have been greatly reduced by agricultural practices. However, the activities of humans have also created new habitats, such as idle bluegrass pastures and grassy, unmowed roadsides (Getz, 1985). In fact, there is evidence that the use of grassy roadsides allowed a significant expansion of the range of meadow voles into the high intensity agricultural region of central Illinois (Getz, 1985).

Microtus are induced ovulators, with copulatory activity being the most effective stimuli for the induction of ovulation (Sawrey & Dewsbury, 1985). Further aspects of the reproductive biology of Microtus, such as female reproductive physiology, and its responsiveness to male-produced chemosignals, will be discussed in greater detail in subsequent chapters.

Table 1 - 1. Some Differences Among Montane, Meadow, and Prairie Voles.

Characteristic	Montane voles	Meadow voles
Social organization	Generally polygamous	Generally polygamous
Ejaculation frequency	High	High
Stimulus requirements for pregnancy initiation	High	?
Coolidge effect	Present	Present
Contact proneness	Low	Low
Female response to first male approach	High aggression	High aggression
Male mating with several females	Relatively indiscriminate	?
Male choice re. mated females	Relatively indiscriminate	Relatively indiscriminate*
Female choice re. familiarity	Relatively indiscriminate	?
Female choice re. dominance	Relatively indiscriminate	?

After Dewsbury (1987, 1988a, 1988b). * = Salo (personal communication, May, 1990).

Prairie voles

Often

monogamous

Low

Low

Absent

High

Low

aggression

Concentrate on

a few females

Prefer unmated

females

Prefer male

which copulated

Prefer

dominant male

CHAPTER 2
GENERAL METHODS: SUBJECTS, HOUSING, AND APPARATUS

Montane (M. montanus), meadow (M. pennsylvanicus), and prairie voles (M. ochrogaster) were used as subjects in this dissertation. A total of 209 montane, 19 meadow, and 25 prairie vole males were used. Two-hundred and ninety-one montane, 21 meadow, and 35 prairie vole females were used. Breeding colonies of each of these species were maintained in the Psychology Department at the University of Florida. The montane vole colony was derived from animals received from the University of Utah. The prairie vole colony was derived from animals received from the University of Illinois. The meadow vole colony was derived from animals received from the University of Massachusetts. Efforts were made to avoid inbreeding and maintain genetic diversity in all colonies. Animals paired for breeding did not have common grandparents.

All animals were maintained in windowless, air-conditioned rooms on reversed 16:8 light-dark photoperiods with lights going out at 1200 hr. All rooms contained both male and female voles. Animals had wood shavings for bedding and water available ad libitum. Prairie and montane voles were maintained at all times on Purina rabbit chow. Meadow voles were raised on Purina rat

chow, but changed to rabbit chow at least one month prior to the start of testing.

Unless otherwise noted, females of all three species were housed with their littermates in 48 x 27 x 13 cm or 29 x 19 x 13 cm plastic tub cages until the time experiments began. Animals housed with littermates occasionally inbreed. These instances were noted and females from these litters were excluded from experiments.

Males used in all experiments were sexually experienced, each having been observed to copulate prior to testing. Males were housed individually in 29 x 19 x 13 cm cages when not in experiments.

Different animals were used in the estrus induction experiment (Chapter 3) and the pregnancy maintenance experiments (Chapter 4). The experiential history of males and females used in the estrus induction experiment is described in Chapter 3. In pregnancy maintenance experiments (Chapter 4) different females were used for each experiment. Males, with the exception of Experiment 1, were never used more than once in an experiment, but were sometimes used in more than one experiment. Males used in Experiments 1, 2, and 3 were drawn from a pool of 75 animals. Males used in Experiments 4 and 5 were drawn from a different pool of 108 animals. Males were always allowed at least 1 week recovery after tests where copulation occurred. Further details regarding experiential history are provided in Chapter 4.

In both estrus induction (Chapter 3) and pregnancy maintenance (Chapter 4) experiments subjects were tested in 48 x 27 x 13 cm clear polycarbonate cages. Each cage was equipped with a removable wire mesh partition that divided the cage in half. Openings in the mesh measured approximately 7 x 7 mm. The mesh prevented extensive behavioral interactions but allowed any chemosignals to pass freely. Testing occurred in the dark phase of the light-dark cycle. Illumination during testing was provided by either a 7 watt white bulb, or a 25 watt white bulb shielded to dampen illumination to approximately a 7 watt level.

CHAPTER 3
ESTRUS INDUCTION IN *MICROTUS*

Introduction

This chapter is concerned with the induction of estrus in montane, meadow, and prairie voles. Females of each species were compared with respect to vaginal cytology and the duration of male exposure needed for receptivity. Species differences in the responsiveness of female reproductive physiology were examined, and a framework was developed to explain these differences as they relate to differences in social organization and mating systems.

Male-Induced Estrus

One familiar aspect of the reproductive biology of rodent species commonly used in laboratory research (e.g., rats, mice, hamsters) is the estrous cycle observed in adult females that are housed in isolation. Cyclic changes occur in the internal hormonal milieu which result in periods of sexual receptivity (behavioral estrus) and periods of nonreceptivity. The endocrine activity of the female's system is mirrored by changes in the vaginal epithelia. Changes in the vaginal epithelia can be monitored with the vaginal smear technique and used to predict when the female will be behaviorally receptive (Feder, 1981; Nalbandov, 1976).

In contrast, female Microtus are induced ovulators and do not display regular cyclical changes in the vaginal epithelia when housed in isolation. Stimuli from the environment, particularly male-related stimuli, are thought to induce endocrine changes that promote sexual receptivity (see Sawrey & Dewsbury, 1985).

Species Differences in Female Reproductive Physiology

Not all species of Microtus have received equal study. Some have been studied rather extensively, whereas very little is known about others. At issue is the generality of knowledge gained with one or a few Microtus species to Microtus as a whole. In some cases generalities appear valid. For example, there appear to be broad similarities across species in certain aspects of female reproductive physiology (e.g., induced ovulation, lack of estrous cyclicity, postpartum estrus; see Richmond & Stehn, 1976).

At a finer level of analysis, however, information on female reproductive physiology may be less universal. For example, prairie voles have received the most study of Microtus species with respect to the influence of male-produced chemosignals on estrus induction (e.g., Carter et al., 1980; Carter et al., 1987; Richmond & Conaway, 1969). Effects of male contact and the resultant physiological changes have been investigated. Some authors have generalized this work to other species. For example, Heske et al. (1988) have proposed a "general reproductive

syndrome" (p. 1154) for Microtus based on research conducted with prairie voles.

Other evidence indicates that important species differences may exist in female reproductive physiology and responsiveness to male-produced chemosignals. Sawrey and Dewsbury (1985), in an comprehensive review, noted that species appear to differ in the proportions of cell types found in vaginal smears. Recall that endocrine activity is thought to be mirrored by changes in the vaginal epithelia. Females of some species show high levels of vaginal cornification only after direct exposure to males or their chemosignals; when not exposed to males, a heavy invasion of leukocytes is present in vaginal smears. Females of other species may show high levels of vaginal cornification in the absence of direct male contact (see Table 3-14). Additionally, there is suggestive evidence that species may differ in the type and duration of male contact needed for behavioral receptivity (see Table 3-14).

Species Differences in Female Reproductive Physiology and Mating Systems

The fundamental premise for the work reported in this chapter is that species differences in estrus induction among Microtus exist and may have important implications for the emergent social organization and mating systems of those species. There may be two distinguishable types of estrus induction systems present among mature Microtus females of different species. The first type, referred to as

male-dependent estrus, is characterized by the necessity of the female being exposed to stimuli from males for a relatively long period of time before estrus is attained. Without direct male contact the female usually remains in a "diestrous" condition evidenced by the predominance of leukocytes over other cell types in vaginal smears and the lack of behavioral receptivity towards males. When exposed to stimuli from males, females with this type of physiology show relatively reliable and predictable increases in vaginal cornification and an increased likelihood of behavioral receptivity.

The second type of estrus induction system is referred to as male-independent. Species of this type do not attain estrus totally independent of male stimulation, but rather are, relative to male-dependent species, much less dependent on direct male contact. Females of species with this type of system may show an "estrous" smear (i.e., predominantly cornified cells present) and may be receptive to the sexual advances of a male without prior direct contact.

Levin and Johnston (1986) have proposed that priming effects due to the presence of conspecifics are more likely to be found in social species rather than more solitary species. The value of such capacities would be more relevant to social species, as the presence of conspecifics and their chemosignals are more likely to be an important and pervasive part of the environment. In the context of the male-dependent versus male-independent distinction

described above, one would expect male-dependent species to be more social. Thus, the degree of responsiveness to chemosignals in a given species should be related to the social organization and mating system displayed by that species. This possibility can be tested in Microtus, as species appear variable in responsiveness to chemosignals, and social organization and mating systems.

In male-dependent species, males and females would be expected to be more often in close contact, perhaps co-nesting, as an extended duration of male contact is needed for estrus induction in the female. This gregariousness may be associated with the development of monogamy and may be manifested in extended family groups living in the same nest area, or a pairbond between a male and female. If females need an extended duration of male contact before estrus induction occurs, males may be limited to the number of females they can induce, thus being more likely to mate monogamously.

Male-independent species, on the other hand, may be expected to be less gregarious as far as male-female relations (e.g., no co-nesting), as extended periods of male-female contact are not needed for estrus induction. In these species males are not as constrained by the female requirements for estrus induction, and, other factors being equal, may have the opportunity to fertilize multiple females. Thus, male-dependent estrus attainment may be associated with social species having a monogamous mating

systems, whereas male-independent estrus may be associated with asocial species having polygamous mating systems.

Methodological Concerns in Studying Male-Induced Estrus

To determine if there are two distinguishable types of female reproductive physiology among Microtus species (i.e., male-independent and male-dependent), specific information must be obtained on 1) the duration of male exposure needed for behavioral receptivity, 2) vaginal cytology, and 3) the relationship of changes in vaginal cytology and behavioral receptivity. There are a number of important methodological concerns to be addressed if such information is to be obtained.

First consider information on the duration of male exposure needed for receptivity. Some information is available. For example, work from different laboratories indicates that female prairie voles need more than 24 hr of male exposure for behavioral receptivity (Carter et al., 1987; Richmond & Conaway, 1969), whereas montane voles may copulate after less than 24 hr of exposure (Sawrey, 1989). Different methods used in different laboratories have left a myriad of variables uncontrolled, however, and make effective species comparisons impossible.

In making species comparisons, laboratory procedures, such as cage changing regimens, need to be specified. These have been shown to affect a variety of reproductive processes (Clulow et al., 1982; Ferguson et al., 1987; Rohrbach, 1982) including vaginal cytology (Richmond &

Conaway, 1969). Changes in vaginal cytology are thought to be correlated with behavioral receptivity (Feder, 1981; Nalbandov, 1976; see also Adler & Bell, 1969), and procedures that cause changes in vaginal cytology may also result in changes in the duration of male exposure needed for receptivity.

The housing conditions of animals before they are exposed to stimuli from males must be considered. For example, Jannett (1980) housed montane vole females together in groups of four or five before they were exposed to males. Sawrey (1989) suggested that differences between his observations of reproductive function in montane voles and the results Jannett obtained could be due to such housing procedures, in that housing females in groups is known to suppress reproductive function (van der Lee & Boot, 1955).

Another factor to be controlled is the time individual animals have been separated from their littermates. In some species of Microtus littermates suppress growth and reproductive maturation of one another when housed in the same cage (Carter et al., 1986; Batzli et al., 1977). Thus, females may vary in their degree of reproductive suppression dependent on how long they have been separated from littermates.

Stimuli for estrus induction can be provided by one (e.g., Richmond & Conaway, 1969; Witt et al., 1988), or more than one male (Hofmann & Getz, 1988). In some cases the origin of stimuli (i.e., from one or more males) is not

fully clear (e.g., Carter et al., 1980; Carter et al., 1987). Jemiolo (1987), based on work with European pine voles (Pitymys subterraneus), suggested that the stimulation from a succession of males may be more effective than constant stimulation from one male.

A related issue concerns the male used for behavioral testing. The male can be the inducing male, or a different sexually experienced male used only for receptivity testing. The latency to mating may be influenced by the familiarity of the pair.

The sexual experience of the male may be an important factor. Witt et al. (1988) found that naive prairie vole males had a greater median latency to mate than experienced males (52 hr vs. 41 hr). Although this difference was not statistically significant, the sample sizes were small.

The actual modalities of male exposure can vary widely. Females can have full contact with males (e.g., Witt et al., 1988), or be exposed to males from behind a mesh barrier (Carter et al., 1980; Richmond & Conaway, 1969), or exposed to their urine (Dluzen et al., 1981), or a combination of direct exposure followed by exposure to olfactory cues (e.g., soiled bedding) (Carter et al., 1987). Although never systematically investigated, varying degrees of contact may influence the duration of male exposure needed for estrus induction, especially since mutual grooming is thought to be important in the process of reproductive activation (Witt et al., 1988). The modality of male

stimulation has been shown to be important in the reproductive activation of house mice. Male urine alone can accelerate puberty in female house mice, but concurrent tactile stimulation from males increases the effect (Bronson & Maruniak, 1975; Drickamer, 1974).

Different aspects of the females' reproductive history may influence the duration of male exposure needed for receptivity. Parity has been shown to alter a variety of reproductive processes (e.g., Clulow et al., 1982; Ferguson et al., 1987; Stehn & Jannett, 1981). Parous montane vole females may be more likely to be receptive than virgins when exposed to stimuli from males (Taylor, personal observation). The number of times a female has been exposed to stimuli from males may also be important. A virgin female that has been exposed to males several times but has not copulated may need less stimulation than a female that has not been exposed to males.

Another consideration is the observation technique. A straight-forward technique for determining the duration of male exposure needed for receptivity is to observe animals from the time of the initial pairing. This is not practical if females need long durations of male exposure. Witt et al. (1988) used time-lapse videotape to observe prairie vole pairs. In pilot work for the present studies time-lapse videotape techniques were found unsatisfactory. Clear resolution of behavioral events indicating copulation (e.g., lordosis, mounting, intromission, etc.) was not possible.

Pierce et al. (1988) have argued that it is difficult to discern the various aspects of copulatory behavior (e.g., mounts, intromissions, and ejaculations) with a videotape analysis. Sawrey (1989) checked for sperm in vaginal smears and counted back a gestation period from the date of birth of a litter in newly paired montane voles to determine the duration of male exposure needed. However, there are also problems with this method. For example, sperm may not be present in the reproductive tract of females that have copulated (see Austin & Dewsbury, 1986), and animals that have copulated do not always give birth. Additionally, gestation periods may vary in length. For example, Richmond and Conaway (1969) found prairie vole gestation periods to vary from 20.5 to 23 days in one experiment and observed interlitter intervals in breeders as short as 19 days.

Finally, different laboratories working with the same species may obtain different rates of estrus induction. For example, Carter et al. (1987) found approximately 70% of virgin female prairie voles attained receptivity. Although not specifically recorded, in work in this laboratory using male-induced nulliparous prairie voles (Taylor & Dewsbury, 1988), the proportions of females attaining estrus were substantially lower (Taylor, personal observation). Conditions in different laboratories (e.g., temperature, humidity, diet, noise and disturbance levels) may result in females of the same species being at different "baselines"

with regard to reproduction, which in turn could alter the duration of male exposure needed for estrus induction.

Information on species differences in vaginal cytology would also be useful. Examination of vaginal cytology may prove to be a reliable way to classify species, as male-dependent and male-independent species should show different levels of cornification when not directly exposed to males. Sawrey and Dewsbury (1985) have noted that information on vaginal smear cellular composition often is available, but precise detail regarding the conditions under which smears were obtained, and the means for determining cellular composition, is often lacking. Since relatively simple environmental disturbances (e.g., cage changes) can alter vaginal cytology (Richmond & Conaway, 1969), and different methods have been used to assess smear contents, effective species comparisons are impossible with the present data base. There have been few attempts to study the relationship of changes in vaginal cytology and behavioral receptivity in any Microtus species (Sawrey & Dewsbury, 1985).

Estrus Induction in Montane, Meadow, and Prairie Voles

It is proposed that two types of female reproductive physiology are present among different species of Microtus (male-dependent and male-independent), and these physiological types are related to the emergent social organization and mating system of the species in question.

In the following study, montane, meadow, and prairie voles were compared in an effort to evaluate this hypothesis.

These three species are suitable for comparison as they appear to differ in social organization and mating systems. Field and laboratory evidence have been accumulating for some time that prairie voles often display a monogamous mating system, with pairbonding and co-nesting being observed (e.g., Getz & Hofmann, 1986; Shapiro et al., 1986; Thomas & Birney, 1979). Montane voles are thought to be asocial and possess a polygynous mating system (Jannett, 1980, 1982). Meadow voles are thought to be asocial and possess a promiscuous mating system (Madison, 1980a, 1980b).

These species may also differ in female physiology. While there have been relatively few studies of estrus induction per se, information can be gleaned from studies conducted with these species on a variety of topics pertaining to reproduction. What follows is a review of the information on estrus induction that is available for each of these species. Females in studies described below are nulliparous unless otherwise noted.

Prairie voles

Male-induced estrus has received considerable study in prairie voles. A consistent finding is that prairie voles need at least 24 hr of male exposure for behavioral receptivity. Richmond and Conaway (1969) found that females were usually behaviorally receptive after 2 - 6 days of being housed adjacent to a male. More recently, Carter et

al. (1987) found that physical contact with a male for 1 hr or more, followed by 30 or 48 hr of exposure to male soiled bedding, resulted in 60 - 70% of females attaining behavioral receptivity. Only 29 - 37% of females receiving 1 or 18 hr of male contact and then placed in a clean cage were found to be receptive. Witt et al. (1988), using a time-lapse video technique, found a latency to mate of 27 - 68 hr in undisturbed pairs. The median latencies to mate were 52 hr in pairs with naive males and 41 hr in pairs with sexually experienced males.

Prairie voles generally show leukocytic smears in isolation, with proximity of a male effectively inducing cornification. Richmond and Conaway (1969) found that whereas 98% of female prairie voles housed away from males displayed persistent anestrus and imperforate vaginas, 71% of females housed across a wire mesh barrier from a male showed vaginal cornification. The relationship between changes in vaginal epithelium, as indicated by vaginal smear patterns, and behavioral receptivity, has not been extensively investigated. Richmond and Conaway (1969) reported that prairie vole females with greater than 50% cornified cells in a smear were usually receptive to males and that females with as few as 10% cornified cells were occasionally found to be receptive.

Montane voles

Montane voles have received less study with respect to the duration of male exposure needed for receptivity.

Sawrey (1989) attempted to determine the latency to copulation in newly paired montane voles couples by searching for sperm in vaginal smears and counting back a gestation period from the date of birth (see Methodological Concerns in Studying Male-Induced Estrus). He concluded that 5 of 18 females copulated within 12 hr of pairing (based on vaginal smears), and that 5 more females copulated within 24 hr of pairing based on either vaginal smears or the date a litter was born. Three more females copulated between 48 hr and 15 days of pairing, and in 5 females copulation was never detected.

The vaginal cytology of montane voles has been studied in detail. Montane voles can show smears that consist of predominantly cornified cells in the absence of direct male contact (Sawrey, 1989; Shapiro, 1987). Sawrey (1989) housed 14 montane voles individually in a colony room and took daily vaginal smears for 30 days. The most common smear type observed consisted of large numbers of both cornified and leukocyte cells (about 60% of all smears). About 30% of all smears were predominantly cornified, consisting of at least 70% cornified cells. Very few smears were found to consist of predominantly leukocytes (about 2%). Direct exposure to males results in greater vaginal cornification (Jannett, 1980; Sawrey, 1989). The validity of smears for predicting behavioral receptivity in montane voles has not been assessed however.

Meadow voles

Meadow voles have received much less study than prairie or montane voles with regard to estrus induction. From the study of ovulation by Lee et al. (1970), one can infer that parous female meadow voles copulated within 24 hr of male exposure. The duration of male contact necessary for behavioral receptivity in nulliparous females has not been determined.

Meadow voles are known to show vaginal cornification in the absence of direct male contact. Clulow and Mallory (1970) took daily vaginal smears on 40 females for 20 days. Thirty females showed predominantly cornified cells for the entire period, 8 showed irregular leukocytic invasion of predominantly cornified cells, and 2 animals showed constant leukocyte predominance for the whole period. Exposure to adult males or their urine has been shown to increase vaginal cornification (Baddaloo & Clulow, 1981). The relationship between vaginal cornification and behavioral receptivity has received little investigation. Grey et al. (1977) reported that meadow voles that had been male exposed and had at least 50% cornified cells in a vaginal smear had "a good probability of showing receptive behavior"(p. 1310), but more specific information is not available.

Thus, there is some evidence suggestive of species differences in the duration of male exposure needed for receptivity among these species. This evidence must be qualified, however, by considerable methodological

differences and a general lack of study directed specifically at the process of estrus induction.

There is also evidence suggestive of differences in species characteristic vaginal cytology. Again this evidence must be qualified considering the possibility of environmental factors influencing vaginal cytology and the methodological differences of various studies. There has been little systematic work relating vaginal smear cellular composition and behavioral receptivity.

Experimental Plan

The following experiment was designed to compare directly montane, meadow, and prairie voles with respect to the estrus induction process while avoiding as many of the above described methodological pitfalls as possible. In this two part experiment species were compared regarding 1) species characteristic vaginal cytology, 2) the duration of male exposure needed for receptivity, and 3) the relationship of changes in vaginal cytology and behavioral receptivity. In Part A nulliparous females were studied; parous females were studied in Part B.

In order to effect this comparison, a variety of measures of the estrus induction process were taken. Behavioral measures included the proportion of females that were induced to behavioral receptivity by exposure to males, and the duration of male exposure needed for behavioral receptivity. A quantitative analysis of species differences in vaginal cytology was conducted. The validity of vaginal

smears as predictors of behavioral receptivity was investigated by comparing smears taken from animals that did and did not copulate.

With the available literature on social organization and mating systems, and female reproductive physiology for each species, several predictions were generated. It was predicted that montane and meadow voles would be rather similar with respect to the estrus induction process and would conform to the male-independent physiological type. Prairie voles were expected to conform to the male-dependent physiological type. Thus, montane and meadow voles were expected copulate with a shorter duration of male exposure than prairie voles. It was also expected that montane and meadow voles might copulate without prior direct contact with males, whereas this would not occur in prairie voles. Finally, montane and meadow voles were expected to show a greater degree of vaginal cornification before being exposed to males than prairie voles.

The study of the effects of parity on reproductive processes deserves more consideration than it has received. Parity has been shown to affect reproductive processes in voles (e.g., Clulow et al., 1982; Ferguson et al., 1987; Stehn & Jannett, 1981) under various circumstances. Parous females were studied in this work to establish the generality of species differences in estrus induction. Predictions were not clear-cut. It was expected that species differences in the duration of male exposure needed

for receptivity would remain, although the magnitude of the differences might change. The main expectation, based primarily on previous personal observations in the laboratory, was that a greater proportion of parous than nulliparous females would be induced.

Method

Subjects

In Part A nulliparous females were used. Subjects were 22 montane vole females (86 - 120 days of age), 35 prairie vole females (114 - 140 days of age), and 21 meadow vole females (115 - 159 days of age). Prairie vole females were tested in two separate groups, referred to as squad I (n = 23) and squad II (n = 12). Squad I prairie vole females were tested concurrently with montane voles. Squad II prairie voles were tested concurrently with meadow voles.

In Part B parous females were used. Parous females were obtained by pairing 12 montane, 20 meadow, 15 squad I prairie, and 12 squad II prairie vole females that were used in Part A with males. Ten montane, 13 meadow, 12 squad I prairie, and 9 squad II prairie vole females that produced litters were used as subjects in Part B. Litters were taken from females shortly after birth and females were tested no earlier than 10 days (generally 2 - 3 weeks) after the pups were taken. Females were housed individually (rather than in littermate groups) prior to the start of the experiment, but other housing and maintenance conditions were identical to those for Part A.

Twenty-six male montane voles, 25 male prairie voles, and 19 male meadow voles were used; all were sexually experienced. Some males were used in both Parts A and B, but were never paired with the same female. Montane and prairie voles were tested in the same room. Meadow voles were tested in a different room that contained both montane and prairie voles.

Apparatus

Subjects were tested in 48 x 27 x 13 cm clear polycarbonate cages with removable wire mesh dividers.

Procedure

In both Parts A and B experimental procedures could continue for up to seven days for each animal. Data collection was terminated for a female if she was observed to copulate. All females in Parts A and B underwent both vaginal smearing and behavioral receptivity testing.

Vaginal smears

Females were taken from their littermate groups (individual cages in Part B) and housed individually in clean 29 x 19 x 13 cm cages with fresh bedding (Day 0). Beginning the next morning vaginal smears were obtained with a thin wire loop and tap water (Day 1). Smears were taken each morning and continued for seven days (until Day 7) unless the female copulated in a behavioral test. No further smears were taken if the female copulated. Smears were fixed in 95% alcohol, stained with toludine blue, and examined under a microscope after drying. A view

representative of the overall proportions of cell types was found, and the numbers of cornified, nucleated, leukocyte cells were counted and converted to a percentage of the total number of cells. In this manner, an estimate of the percentage of cornified, nucleated, and leukocyte cells was obtained.

Vaginal smears also were categorized based on the percentage of cornified cells present. Below are the categories used:

0 - Zero to 19% cornified cells.

2 - Twenty to 39% cornified cells.

4 - Forty to 59% cornified cells.

6 - Sixty to 79% cornified cells.

8 - Eighty to 100% cornified cells.

NUC - Greater than 15% nucleated cells. Receiving a "NUC" rating did not exclude smears from the categories based on percentage of cornified cells present. This category exists to give an indication of how often nucleated cells are present in a substantial number.

SP - These smears are classified as "sparse" as there were not enough cells present in any field of view to make an accurate estimate of the percentages of different cell types.

Behavioral testing

Behavioral testing took place in the afternoon and began two days after vaginal smears were begun (Day 3). Behavioral testing could continue for five days (Days 3 -

7), but was terminated if the female was observed to copulate. On the first day of behavioral testing (Day 3) each female was placed in one half of a divided cage and a sexually experienced conspecific male placed in the other half. After a 15 min habituation period the divider was removed and the animals were allowed to interact for 1 hr. The divider was then replaced to separate the animals. Each male and female remained in this same divided cage for the remainder of the experimental procedure (Days 3 - 7). The divider was removed for 1 hr each day to allow full interaction until copulation was observed, or the experimental procedure came to an end (Day 7). The divider was always removed during the dark phase of the light-dark cycle at approximately 1500 hr. The primary measure recorded was the day of behavioral estrus, as indicated by the occurrence of copulatory behavior. The latency to the onset of copulatory behavior (to the nearest min) within each 1 hr test was also recorded.

Statistical Analysis

Nonparametric statistics were used throughout. Statistical comparisons were only made when sample sizes were four or greater. Species were compared with Kruskal-Wallis multiple group tests. Pairwise comparisons were conducted using Mann-Whitney U tests. Other statistical techniques used are identified in the text.

Results

Results are presented under four headings to enhance clarity.

Copulatory Behavior

In Part A (nulliparous females), 14 of 22 montane voles, 2 of 21 meadow voles, 8 of 23 squad I prairie voles, and 3 of 12 squad II prairie voles attained behavioral receptivity. There were significant species differences in the proportion of females attaining receptivity (Fisher Exact Test; see Table 3-1). The proportion of copulating females was greater in montane than in prairie (squads I and II) or meadow voles. Proportionally fewer meadow voles copulated than squad I prairie voles, but not squad II prairie voles.

In Part B (parous females), 8 of 10 montane voles, 8 of 13 meadow voles, 9 of 12 squad I prairie voles, and 4 of 9 squad II prairie voles attained receptivity. There were no significant differences in the proportions of females attaining behavioral receptivity (Table 3-1).

In Part A species differences were found in the temporal distributions of behavioral receptivity. Comparing females that attained behavioral receptivity revealed that species differed with respect to the day on which copulation occurred [H (3, N = 27) = 11.70, $p < .01$]. Montane voles that attained behavioral estrus did so significantly earlier than squad I prairie voles (U = 13.00, $p < .005$). Since so few meadow voles (N = 2) and squad II prairie voles (N = 3)

copulated, statistical pairwise comparisons were not conducted. See Figure 3-1 and Table 3-2 for the number of animals in each group that copulated on each day.

Temporal distributions of behavioral receptivity also differed among species in Part B [H (3, N = 29) = 15.55, p < .005]. Pairwise comparisons indicated that both montane voles and meadow voles that attained behavioral estrus did so significantly earlier than squad I prairie voles (montane vs. squad I prairie, U = 13.50, p < .05; meadow vs. squad I prairie, U = 1.50, p < .005). Meadow voles, but not montane voles, attained behavioral receptivity significantly earlier than squad II prairie voles (meadow vs. squad II prairie, U = 0.00, p < .01; montane vs. squad II prairie, U = 6.00, n.s.). The montane versus squad II prairie vole comparison approached significance however (p = .0854). Montane and meadow voles did not differ significantly (U = 29.00, n.s.). Prairie voles of squads I and II did not differ significantly (U = 18.00, n.s.). Figure 3-2 and Table 3-2 show the number of animals in each group that copulated on each day.

Some females were observed to copulate without being previously exposed to stimuli from males (i.e., females copulated on Day 3, the first day of male exposure; see Table 3-2). In Part A, 3 of the 14 montane voles that copulated did so on the first day of male exposure. In Part B, 5 of 8 montane and 5 of 8 meadow voles that copulated were observed to copulate on the first day of male exposure.

Prairie voles were never observed to copulate without prior male exposure.

In Part A, within 1 hr tests in which copulation did occur, montane voles had a significantly greater latency to copulation than squad I prairie voles ($U = 7.5$, $p < .001$; Table 3-3). Meadow voles and squad II prairie voles were not included in the statistical analysis because of small sample sizes. There were no significant differences in the latency to copulation within 1 hr tests in Part B (Table 3-3).

Vaginal Smears of Isolated Females

To assess species differences in the percentages of cell types in vaginal smears before male exposure, the percentages of cornified, nucleated, and leukocyte cells were compared across all four groups for Days 1, 2, and 3. These analyses were conducted for Parts A (nulliparous females) and B (parous females). Smears that were classified as sparse (SP) were not included in the statistical analyses.

In Part A, for Day 1, significant species differences were found for the percentages of cornified cells [$H (3, N = 64) = 16.39$, $p < .005$], nucleated cells [$H (3, N = 64) = 17.99$, $p < .001$], and leukocytes [$H (3, N = 64) = 14.41$, $p < .005$]. Means and results of the pairwise comparisons are presented in Table 3-4. For Day 2, significant species differences were found for the percentages of cornified cells [$H (3, N = 71) = 23.95$, $p < .001$] and leukocytes [H

(3, N = 71) = 26.31, $p < .001$] but not for nucleated cells [H (3, N = 71) = 2.08, n.s.]. Means and results of the pairwise comparisons are presented in Table 3-5. For Day 3, significant species differences were found for the percentages of cornified cells [H (3, N = 66) = 18.05, $p < .001$] and leukocytes [H (3, N = 66) = 18.76, $p < .001$] but not for nucleated cells [H (3, N = 66) = 0.38, n.s.]. Means and results of the pairwise comparisons are presented in Table 3-6. Actual values of U for the pairwise comparisons for Days 1, 2, and 3 are presented in Appendix A.

In Part B, for Day 1, significant group differences were found for percentages of cornified cells [H (3, N = 42) = 15.32, $p < .005$] and leukocytes [H (3, N = 42) = 14.47, $p < .005$], but not for nucleated cells [H (3, N = 42) = 4.27, n.s.]. Means and results of the pairwise comparisons are presented in Table 3-7. For Day 2, significant group differences were not found for cornified cells [H (3, N = 43) = 6.98, n.s.], nucleated cells [H (3, N = 43) = 1.92, n.s.], or leukocytes [H (3, N = 43) = 7.37, n.s.]. However, pairwise comparisons were conducted for percentages of cornified cells and leukocytes since the values obtained approached significance ($p = .0713$ and $p = .0598$ respectively). Means and results of the pairwise comparisons are presented in Table 3-8. For Day 3, significant group differences were found for percentages of cornified cells [H (3, N = 43) = 10.10, $p < .05$] and leukocytes [H (3, N = 43) = 10.41, $p < .05$], but not for

nucleated cells [H (3, N = 43) = 0.90, n.s.]. Means and results of the pairwise comparisons are presented in Table 3-9. Actual values of U for all pairwise comparisons are presented in Appendix A.

In Appendix B, vaginal smears for Days 1, 2, and 3 of parts A and B are categorized based on degree of vaginal cornification and other criteria as described above (see Vaginal smears).

Vaginal Smears From Females That Copulated

Further analyses were conducted with smears from females that copulated. Full statistical analysis was not always possible with this data set. For some comparisons too few animals copulated (in Part A only 2 meadow and 3 squad II prairie voles copulated) and for other comparisons too many animals copulated to allow comparison of animals that did and did not copulate (in Part B only 2 montane and 3 meadow voles did not copulate).

Smears taken from animals of each species on the day of copulation were compared with respect to the percentages of different cell types. In Part A, smears from squad I prairie voles did not differ from montane voles with respect to cornified cells (U = 31.50, n.s.) or leukocytes (U = 34.50, n.s.), but squad I prairie voles did have a greater percentage of nucleated cells (U = 22.50, $p < .05$; Table 3-10). No statistical tests were conducted with meadow voles and squad II prairie voles; means are presented in Table 3-10. In Part B there were no significant differences among

all four groups of animals in percentages of cornified cells [H (3, N = 29) = 0.73, n.s.], nucleated cells [H (3, N = 29) = 6.04, n.s.], or leukocytes [H (3, N = 29) = 1.52, n.s.] in smears taken on the day of copulation (Table 3-10).

Smears of animals that did copulate were compared to smears from animals that did not copulate. Within each species, the percentage of cornified cells in smears for the day of copulation was compared to the day in which females that did not copulate had their highest cornified cell percentage when mating was possible (Days 3 - 7). In Part A no significant differences were found in the smears of montane (U = 42.00, n.s.) or squad I prairie vole smears (U = 33.50, n.s.; Table 3-11) that did or did not copulate. No statistical tests were conducted with meadow and squad II prairie voles; means are presented in Table 3-11. In Part B meadow voles (U = 9.0, n.s.) and squad II prairie voles (U = 8.0, n.s.) that did and did not copulate did not differ significantly (Table 3-11). No statistical tests were conducted with montane and squad I prairie voles; means are presented in Table 3-11.

Smears taken on the day of copulation were compared to smears taken from the same animal the day before copulation (Wilcoxon test). This analysis was conducted only with animals having at least two days when copulation was possible (i.e., the male had access to the female during the 1 hr behavioral receptivity tests); animals that copulated on the first day of male exposure were excluded. In Part A,

montane voles showed a significant increase in vaginal cornification from the day before to the day of copulation ($T = 3.50$, $p < .01$), but squad I prairie voles did not ($T = 8.50$, n.s.; Table 3-12). No statistical tests were conducted with meadow and squad II prairie voles; means are presented in Table 3-12. In Part B, squad I prairie voles showed a significant increase in vaginal cornification from the day before to the day of copulation ($T = 2.00$, $p < .01$). The value for the squad II prairie voles approached significance ($T = 0.00$, $p = .0643$,; Table 3-12). No statistical tests were conducted with montane and meadow voles; means are presented in Table 3-12.

Smears from each female that did not copulate on the first day of male exposure, but did eventually copulate, were ranked with respect to degree of vaginal cornification (see Appendix C).

In Appendix D, vaginal smears taken on the day of copulation in Parts A and B are categorized based on degree of vaginal cornification and other criteria as described above (see Vaginal smears).

Effects of Parity

Several analyses were undertaken to determine the effects of parity on the duration of male exposure needed for receptivity and vaginal cytology.

The influence of parity on the duration of exposure needed for receptivity was assessed by comparing, in animals that copulated in both parts A and B, the duration of male

exposure needed when nulliparous, and when parous (Wilcoxon test). Meadow voles (n = 2) and squad II prairie voles (n = 1) were not included as the sample sizes were too small. Parity had no effect on the duration of male exposure needed for receptivity in montane [T (N = 8) = 12.0, n.s.] or squad I prairie voles [T (N = 7) = 6.0, n.s.].

For females that were tested in both Part A and Part B, it was possible to compare the proportion of females attaining behavioral receptivity when nulliparous and parous using the McNemar test of changes (see Conover, 1980). Table 3-13 shows for each species the proportions of nulliparous and parous females copulating when all females are included, the proportions of nulliparous and parous females copulating when only females that were tested in Parts A and B are included, and the results of the statistical analysis (using data from females that participated in both Parts A and B). Note that the magnitude of the differences between the proportion of nulliparous and parous females copulating is reduced when only females tested in Parts A and B are included. Of the four groups of animals, only meadow voles were significantly more likely to attain receptivity when parous than when nulliparous (Table 3-13). If all four groups are lumped, then females are significantly more likely to attain receptivity when parous than when nulliparous (Table 3-13).

The influence of parity on vaginal cytology was also investigated. Wilcoxon matched-pairs tests were used to

compare the percentage of cornified cells for females in each group on Days 1, 2, and 3 when nulliparous and parous. Smears that were classified as sparse (SP) were not included in the analyses. Means and statistical results are presented in Appendix E. There was no evidence of a consistent difference in the percentage of cornified cells in smears when females were nulliparous versus parous. In only 1 of 12 tests was a significant difference found (Day 2, montane voles; see Appendix E).

General Discussion

It was proposed that differences in female reproductive physiology exist among Microtus species. Some species can be described as male-dependent, in which females are highly dependent on direct male contact of substantial duration for induction of vaginal cornification and behavioral receptivity. Other species can be described as male-independent, in which females are much less dependent, relative to the male-dependent type, on exposure to stimuli from males. It was predicted that prairie voles would show characteristics of the male-dependent type, whereas montane and meadow voles would show characteristics of the male-independent type. Results obtained were concordant with predictions. Below the effects of male exposure on behavioral receptivity are discussed in detail. Vaginal smears, and the relationship of vaginal smears changes to behavioral receptivity are also discussed. Finally, species differences in female reproductive physiology are discussed

in terms of species differences in social organization and mating systems.

Male Exposure and Behavioral Receptivity

As predicted, montane and meadow voles did not differ in the duration of male exposure needed for receptivity, and attained behavioral receptivity with a shorter duration of male exposure than prairie voles. In Part B (parous females) this result was clear cut, and is graphically illustrated in Figure 3-2. It can also be argued that the hypothesis was essentially supported in Part A (nulliparous females; Figure 3-1). Montane voles copulated with a shorter duration of male exposure than prairie vole females. Only two nulliparous meadow vole females copulated and thus statistical comparisons could not be made. However, the two meadow vole females that did copulate did so on the second day of male exposure, whereas 92% of prairie vole females (parous and nulliparous) did not copulate until the third day of male exposure or later (see Table 3-2).

The observation that montane and meadow voles may copulate when first exposed to males also reinforces the male-dependent versus male-independent interpretation. This was most evident in Part B (parous females). Sixty-three percent of both montane and meadow voles that copulated did so without previous direct male contact. In Part A, 21% of nulliparous montane voles that copulated did so without previous direct male contact. Neither nulliparous nor parous female prairie voles were ever observed to copulate

without prior direct male contact, and only 8% of prairie voles that copulated did so after one full day of contact.

Animals that copulated when first exposed to males were not completely deprived of male stimulation, as they were housed and tested in mixed-sex colony rooms. Sawrey (1989) found that montane voles showed higher levels of vaginal cornification in mixed-sex colony rooms than when housed in rooms containing only other females. Similar experiments to the present work could be conducted including females totally isolated from male odors (e.g., different room with separate air supply) to determine the effects of colony room stimulation and if montane and meadow voles can attain estrus completely independent of prior male stimulation.

Crews (1987; Crews & Moore, 1986) has stressed the diversity of reproductive patterns in different species. Many species show the "associated reproductive pattern", in which there is a close correspondence between maximal secretion of sex steroids and mating behavior. For example, ovarian activity in female green anole lizards (Anolis carolinensis) is stimulated by a combination of warm temperatures and courtship displays of the male. The stimulation of ovarian activity leads to sexual receptivity; at other times the female is not receptive (Crews, 1987). Other species may show the "constant reproductive pattern", in which a high level of reproductive readiness is maintained at all times. Both male and female zebra finches (Taeniopygia guttata castanotis) maintain gonadal activity

throughout long periods of drought. Rainfall initiates reproductive behavior, with copulation occurring within minutes of the onset of rains (see Crews, 1987, and references therein).

Perhaps there is a similar variability of reproductive patterns among Microtus species. Montane and meadow voles under these test conditions exhibit characteristics of the constant reproductive pattern. The rapid onset of mating and the high levels of vaginal cornification (see below) may indicate that females maintain a high level of reproductive readiness. Prairie voles, on the other hand, seem to show characteristics of the associated reproductive pattern. Females become sexually receptive only after a period of direct exposure to males that stimulates endocrine activity (Carter et al., 1980; see below). Crews (1987; Crews & Moore, 1986) stresses that fundamental differences are likely to exist in the neuroendocrine mechanisms underlying different reproductive patterns. This would seem unlikely among closely related Microtus species. Perhaps neuroendocrine mechanisms differ in their sensitivity to estrus-inducing stimuli among vole species. It may be that sensitivity differences in fundamentally similar neuroendocrine mechanisms can result in very different reproductive patterns.

Relation to Previous Work

The results with prairie voles were consistent with other work in which estrus induction has been observed in

this species (Carter et al., 1987; Richmond & Conaway, 1969). Richmond and Conaway (1969) found that prairie voles may copulate after 2 - 6 days of exposure, but noted that the estrus induction usually occurred after 2 or 3 days, or not at all. A similarly uniform response was observed here (see Table 3-2 and Figures 3-1 and 3-2), with 75% of all females that copulated doing so during the 1 hr test after 48 hr of exposure (Day 5). Parous female prairie voles have not been studied previously with respect to estrus induction. The duration of male exposure needed was consistent between nulliparous and parous females; parity did not have a significant effect.

The only previous work on the duration of male exposure needed for estrus induction in montane voles was that of Sawrey (1989). In his sample of nulliparous females he found evidence (vaginal smears and date of birth) that 77% of those that copulated did so within 24 hr. In the present work, combining nulliparous and parous females, 77% of females that copulated did so upon first exposure to males (Day 3), or during the 1 hr test after 24 hr of exposure (Day 4). With the methods used in the present work, a distinction can be made between females that copulated immediately upon exposure to males (i.e., within the first hour), and females that need some duration of direct exposure. Twenty-one percent of nulliparous females, and 63% of parous females that copulated did so upon being first exposed to males. These are the first systematic

observations of montane voles copulating upon first introduction to males without prior direct contact. Although a greater proportion of parous females did copulate when first exposed to males (cf. Figures 3-1 and 3-2), parity did not result in a significant change in the duration of male exposure needed for receptivity.

Little information on estrus induction is available for meadow voles. One can infer from Lee et al. (1970) that parous females may copulate within 24 hr of being exposed to males. In this work parous meadow voles did copulate within 24 hr of male exposure, and that like montane voles, 63% of those that copulated did so within an hour of first being exposed to males. Nulliparous female meadow voles had a very low copulation rate (2 out of 21). One possibility is that the duration of male exposure was not great enough to result in estrus induction in these females. This seems unlikely however. Similarly low induction proportions have been observed in nulliparous meadow voles even when females were exposed to males for longer durations (A. Salo, personal communication, 1990). Novelty seems to be a key factor in the estrus induction process (Richmond & Stehn, 1976; Sawrey & Dewsbury, 1985) and leaving a female across from the same male for longer durations does not provide novelty after the initial introduction. The low proportion of females copulating may be related to the promiscuous mating system of meadow voles (Madison, 1980a, 1980b).

Perhaps exposure to a succession of males would be more effective in inducing estrus (see Jemiolo, 1987).

Proportions of Females Attaining Estrus

There were significant species differences in proportions of females attaining estrus in Part A. Nulliparous prairie and meadow voles showed significantly lower proportions of females attaining estrus than nulliparous montane voles. The 25 and 35% levels observed in the two nulliparous prairie vole groups are lower than the 60 to 70% levels reported by Carter et al. (1987). However, Richmond and Conaway (1969) reported that different populations of prairie voles showed different proportions of females induced, and provided data from one population with an induction level of 25%. The proportions observed in the present study were about as expected based on previous work in this laboratory where male-induced nulliparous females were used. Perhaps the reproductive suppression of being housed with littermates (see Batzli et al. 1977) had more of an effect on prairie voles than montane voles. However, nulliparous meadow voles showed an even lower proportion of females attaining estrus, and they have been reported to show little suppression of reproduction when housed with littermates (Batzli et al. 1977).

An extremely small proportion of nulliparous meadow voles attained estrus. Only 2 of 21 females copulated. Extremely low proportions of nulliparous meadow voles attaining estrus has been noted in other work in this

laboratory (A. Salo, personal communication, 1990). As mentioned above, meadow voles, which possess a promiscuous mating system (Madison, 1980a, 1980b), may need exposure to a number of males for high levels of induction. An interesting experiment would be to compare estrus induction levels in prairie voles (purported to have a monogamous mating system, see Table 3-14) and meadow voles, when exposed to a succession of different males. One might predict that a higher proportion of meadow voles would attain estrus, whereas the normal pattern of estrus induction in prairie voles might be disrupted by the succession of males.

Greater proportions of parous than nulliparous females attained behavioral receptivity in all groups (see Table 3-1). However, to test this difference statistically, only animals tested in both Parts A and B could be compared. With this restricted sample the difference in induction levels between nulliparous and parous females was substantially reduced (see Table 3-13). Only meadow voles show a significantly higher proportion of females attaining estrus when parous than when nulliparous (Table 3-13). If the sample size is increased by lumping all four groups together however, then parous females show a significantly higher level of estrus induction overall (Table 3-13).

The lower levels of induction in nulliparous females may reflect the inclusion of females with improperly functioning reproductive systems, a factor less likely in

females that have demonstrated fertility in the past. Studies of reproduction in voles (e.g., Kirkpatrick & Valentine, 1970; Negus & Pinter, 1966; Richmond & Conaway, 1969) and personal experience indicate that a portion of females never successfully breed. Kirkpatrick and Valentine (1970) suggest the failure to reach estrus and copulate is the most likely cause of breeding failure.

Another possible explanation might be the age of females, as the same females were tested in both the nulliparous, and later in the parous conditions. The effects of age on the proportions of females induced to behavioral receptivity has not been specifically addressed in this study or elsewhere. In general there have been few studies of age related influences on reproduction in Microtus. Carter et al. (1980) found that prairie vole females ranging in age from 20 to 70 days did not differ in male-stimulated reproductive activation (as measured by uterine weight increases). Fertility differs with age in grey-tailed voles (M. canicaudus) (Hagen & Forslund, 1979). Grey et al. (1977) found that older female meadow voles required greater vaginal stimulation than younger females for maximal ovulation rates.

Vaginal Smears

Sawrey and Dewsbury (1985), in a comprehensive literature review, noted that species differences in the proportions of cell types in vaginal smears appeared to exist in Microtus. They warned that conclusions must be

limited however, as the conditions under which animals were studied, and the means for determining the proportions of cell types, are generally not specified.

In the present study, species differences were confirmed when nulliparous and parous females were housed under identical conditions and a uniform quantitative procedure was used to determine the proportions of various cell types. Results were quite consistent in nulliparous (Part A) and parous (Part B) females. In general, meadow and montane voles had greater proportions of cornified and fewer leukocytes in vaginal smears than prairie voles before the animals were exposed to males (Days 1, 2, and 3). The percentages of cell types did not differ in montane and meadow vole smears. These conclusions are supported by inspection of the distribution of smears from Days 1, 2, and 3 into categories in Appendix B. While some significant species differences in nucleated cells were detected in Part A (see Table 3-4), these differences were small in magnitude. Inspection of Appendix B reveals that smears with substantial numbers of nucleated cells were rare in all species. Very few smears were classified as NUC (i.e., greater than 15% nucleated cells). Parity had no significant influence on vaginal cytology of females before male exposure (Appendix E).

The proportions of cell types observed in nulliparous montane vole smears were consistent with those reported by Sawrey (1989) in his studies of nulliparous montane voles.

He found that cornified cells were rarely absent, and most smears consisted of at least 30% cornified cells. He also found that leukocytes were frequently observed in combination with cornified cells, and substantial numbers of nucleated cells were rarely observed. Additionally, Sawrey (1989) found evidence that montane vole smears are more frequently dominated by cornified cells than prairie vole smears when he compared his dissertation data to previously collected prairie vole data. Few details were given however.

Species differences in the characteristic smear types shown by females when not directly exposed to males may be predictive of how rapidly the induction of estrus occurs upon male exposure. In the present work, montane and meadow voles had greater proportions of cornified cells in vaginal smears and responded more rapidly when exposed to males than prairie voles. Perhaps species that show substantial numbers of cornified cells in vaginal smears when not directly exposed to males are physiologically "closer" to estrus (e.g., higher serum estrogen levels) and show the constant reproductive pattern discussed above (see Male Exposure and Behavioral Receptivity).

Vaginal smears may thus be indicative of male-dependence or male-independence. Additional data must be collected with other species to evaluate this hypothesis. Some evidence can be gathered from the literature. For example, field voles (M. agrestis) show cornified smears in

isolation (Breed & Clarke, 1970; Milligan, 1974), and apparently can mate without prior direct contact (Chitty & Austin, 1957). Pine vole (M. pinetorum) smears are dominated by leukocytes in isolation, and they apparently do not mate without several days of direct contact (Lepri, 1986; Schadler & Butterstein, 1979).

Vaginal Smears and Behavioral Receptivity

Sawrey (1989) described the potential association between vaginal smears and behavioral receptivity in Microtus. Contact with males initiates neuroendocrine reflexes that result in increasing levels of serum estrogen (Cohen-Parsons & Carter, 1987). There are two concurrent effects of male exposure and the subsequent elevation of estrogen titers; vaginal cornification increases, and behavioral receptivity becomes more likely (Sawrey, 1989). His scheme offers testable predictions. One might expect 1) receptive females to have high levels of vaginal cornification, 2) receptive females to have higher levels of vaginal cornification than non-receptive females, and 3) increasing vaginal cornification to be associated with behavioral receptivity.

Copulating animals did tend to have smears dominated by cornified cells on the day of copulation (Table 3-7). Interestingly, species differences in the proportions of cell types tended to disappear by the day of copulation. This "convergence" of smear patterns was most evident in parous females, as copulating females of all three species

did not differ in the proportions of any cell types. Prairie voles undergo the greatest change with increased cornified cells and fewer leukocytes. The conclusion that species converge by the day of copulation is reinforced by an inspection of the distribution of smears from the day of copulation into categories (Appendix D). This is suggestive of a smear pattern characteristic of copulation in parous Microtus. Averaging the percentages of cell types of these species reveals a pattern of 60.6% cornified, 1.4% nucleated, and 38.0% leukocytes; values that are consistent with the prevailing notion that high levels of vaginal cornification are associated with behavioral receptivity (Nalbandov, 1976; Sawrey & Dewsbury, 1985). In nulliparous females a similar convergence was present, although only montane and squad I prairie voles could be compared due to sample size constraints. They did not differ in the proportions of cornified cells or leukocytes, mainly due to a large increase in cornification and reduction in leukocytes in prairie voles. A difference of small magnitude was present in proportions of nucleated cells.

If high levels of vaginal cornification are associated with behavioral receptivity, then animals that copulated should have had higher levels of cornification than animals that did not copulate. Within each species, levels of cornification were compared in animals that did and did not copulate. Although this comparison was hindered by sample size in some cases (too few or too many animals copulating

per group), some information is available. No significant differences in level of cornification were found between copulating and non-copulating prairie and montane voles in Part A, and meadow voles and squad II prairie voles in Part B. Thus, the percentage of cornified cells in a vaginal smear does not appear to be a good predictor of behavioral receptivity, at least in the groups tested.

Vaginal cornification might have been a better predictor of receptivity if a new stud had been used for each behavioral test. In the present work, the male was exposed to the same female every day. After being rebuffed by the female before she is receptive, mating attempts may become more infrequent. The male may then fail to copulate when the female does become receptive (and shows a high level of cornification). The experiment discussed above, in which females are exposed to a succession of males, would provide additional information.

Another prediction is that increases in vaginal cornification should be associated with behavioral receptivity. This proposition was tested statistically by comparing smears from the day before copulation to the day of copulation. Additionally, smears were ranked with respect to the degree of vaginal cornification (Appendix C). There are qualifications regarding the application of these analyses to the present data set. The samples are select, in that they are animals that did not copulate on the first day. Also, small sample sizes resulted from too few animals

copulating in a group, or too many animals copulating on the first day. Such analyses are best conducted on species that generally do not copulate upon first exposure to males.

Bearing in mind these qualifications, there was some evidence that an increase in cornification is associated with behavioral receptivity. In Part A copulating montane voles, but not prairie voles, showed a significant increase in the degree of vaginal cornification from the day before to the day of copulation (Table 3-12), and tended to copulate on the day of greatest vaginal cornification (Appendix C). In Part B copulating squad I prairie voles showed a significant increase in vaginal cornification from the day before to the day of copulation; the value for squad II prairie voles approached significance (Table 3-12). Additionally, in Part B females of all species (in this restricted sample) tended to copulate on the day of greatest vaginal cornification, and there appears to be a regular pattern of increase in vaginal cornification in squad II prairie voles and meadow voles (Appendix C).

With the mixed results obtained it is difficult to generate any general statement concerning the predictive validity of vaginal smears with regard to behavioral receptivity. They are certainly not perfect indicators of receptivity, and are perhaps less useful in voles than other species with spontaneous ovulation (e.g., rats, mice) where a cyclic pattern can be ascertained. It appears that in the species studied, females with high levels of vaginal

cornification are more likely to copulate than females with high levels of leukocytic invasion. However, it also appears that high levels of vaginal cornification may be present in the absence of behavioral receptivity. This topic needs further investigation. Perhaps studies with larger sample sizes and different males for inducing and behavioral testing would provide more information.

Female Reproductive Physiology and Social Organization

Interest in Microtus stems largely from studies indicating contrasting patterns of social organization in the field (see Dewsbury, 1987; Table 3-14 references). Differences in mating systems and social organization can be understood as resulting from stable species differences in behavioral tendencies of individuals. Dewsbury (1987, 1988a, 1988b) lists a number of stable differences that may drive differences in mating systems (see Table 1-1). In the present work, species differences in female reproductive physiology were identified in montane, meadow, and prairie voles. These differences in reproductive physiology have behavioral implications, and may contribute to differences observed in social organization and mating systems.

The results presented here are in opposition to the idea of a "general reproductive syndrome" (Heske et al., 1988) for Microtus. Species differences in vaginal cytology are clearly present when females are housed away from males. Species differences are also present in the type and duration of male exposure needed for estrus induction.

Prairie voles appear to conform to what is described herein as the male-dependent class, whereas montane and meadow voles appear to conform to what is described as the male-independent class.

In male-dependent species the opportunity for a male to engage in multiple matings will be limited by the amount of time he must allot for estrus induction. A male-dependent induction system might therefore produce strong pressure for a male to concentrate his reproductive efforts on one female at a time. The results might include co-nesting, pairbonding, and a monogamous mating system. Recent evidence that females can be induced by a series of visits (Hofmann & Getz, 1988) might indicate that males could mate multiply by visiting a number of females several times each, and then mate when they become receptive. However, females induced by repeated periods of male exposure spread over time would still create pressure for a male to focus his mating efforts on one female. A female that a male has visited but left unguarded may become receptive and mate with another male while the original inducing male is absent.

Additionally, a longer period of estrus induction may afford the female greater opportunity for mate evaluation. Vole species with monogamous mating systems generally display mate choice in the laboratory more readily than polygamous species (e.g., Pierce, 1989; Pierce et al., 1989; Shapiro & Dewsbury, 1986; Shapiro et al., 1986). Mate

evaluation may be critical in monogamous species where males contribute substantially to the care of the offspring.

The time constraints imposed by estrus induction would be less in male-independent species, thus allowing males greater opportunity for multiple matings. Females may be readily receptive, or need only a short induction period. Thus, these species may be less gregarious as far as male-female relations (e.g., no co-nesting), and mating systems involving multiple mating by males (polygyny, promiscuity) would be expected.

In the future the association of physiological type and social organization must be examined in additional species to determine its generality. Suggestive information on other species can be gleaned from the literature. In Table 3-14 the evidence is summarized concerning social organization and mating systems, and female reproductive physiology, for six species of Microtus, including those studied here.

Species appear to fall into two groups based on mating system and whether they can be classified as male-dependent or male-independent regarding estrus induction. Prairie voles and pine voles (M. pinetorum) are thought to be social and monogamous, show leukocytic smears in isolation, and require extended male contact for behavioral receptivity. In contrast, montane voles, meadow voles, field voles (M. agrestis), and California voles (M. californicus) are thought to be asocial and polygamous, and are known to show

cornified smears in isolation. There is also evidence that field voles may be receptive without direct male contact. Caution must be exercised at this point however, as the information regarding the duration of male exposure needed for receptivity, and other information on reproductive physiology in pine, California, and field voles has been gathered from a wide variety of sources with many potentially confounding variables uncontrolled.

There appears to be an excellent opportunity for comparative research into female reproductive physiology. Investigation of additional species is necessary to determine if the male-independent versus male-dependent distinction is supported on a wide scale, and if it is associated with the type of social organization displayed by different species. Pine, California, and field voles would seem the best candidates for immediate study, as these species are presently the most studied with respect to social organization and mating system, and female reproductive physiology (see Wolff, 1985; and Sawrey & Dewsbury, 1985). Many other species of Microtus are worthy of study however, as the variability within the genus appears considerable (see Tamarin, 1985).

Table 3-1. Percentages of Females Copulating.

Species	No. cop	% cop	Probability value			
			Mont	Mead	Pr(S1)	Pr(S2)
Nulliparous females						
Mont	14/22	64%	-	0.003	0.05	0.04
Mead	2/21	10%	-	-	0.04	ns
Pr(S1)	8/23	35%	-	-	-	ns
Pr(S2)	3/12	25%	-	-	-	-
Parous females						
Mont	8/10	80%	-	ns	ns	ns
Mead	8/13	62%	-	-	ns	ns
Pr(S1)	9/12	75%	-	-	-	ns
Pr(S2)	4/9	44%	-	-	-	-

No. cop = number of females copulating; % cop = percentage of females copulating; Mont = montane voles; Mead = meadow voles; Pr(S1) = squad I prairie voles; Pr(S2) = squad II prairie voles; ns = not significant ($p > .05$)

Table 3-2. Number of Females Copulating by Day.

		Day of Testing					Did not
Nulliparous	N	3	4	5	6	7	copulate
Montane	22	3	8	2	1	0	8 (36%)
% per day		14	36	9	5	0	
Meadow	21	0	2	0	0	0	19 (91%)
% per day		0	10	0	0	0	
Prairie (S1)	23	0	0	6	2	0	15 (65%)
% per day		0	0	26	9	0	
Prairie (S2)	12	0	1	1	1	0	9 (75%)
% per day		0	8	8	8	0	
Parous							
Montane	10	5	1	1	0	1	2 (20%)
% per day		50	10	10	0	10	
Meadow	13	5	3	0	0	0	5 (39%)
% per day		39	23	0	0	0	
Prairie (S1)	12	0	1	7	0	1	3 (25%)
% per day		0	8	58	0	8	
Prairie (S2)	9	0	0	4	0	0	5 (56%)
% per day		0	0	44	0	0	

Table 3-3. Mean Latency (min) to Copulation in 1 Hr Receptivity Tests.

	Species			
	Mont	Mead	Pr(S1)	Pr(S2)
Nulliparous females				
No. copulating	14	2	8	3
Mean latency (min)	17.0*	23.5	1.4*	46.0
Parous females				
No. copulating	8	8	9	4
Mean latency (min)	11.9	16.3	6.3	3.3

* = significantly different ($p < .001$); Mont = montane voles; Mead = meadow voles; Pr(S1) = squad I prairie voles; Pr(S2) = squad II prairie voles

Table 3-4. Percentage of Cell Types in Vaginal Smears of Nulliparous Females on Day 1.

	Species (column)			
	1	2	3	4
	Mont	Mead	Pr(S1)	Pr(S2)
	(n=20)	(n=18)	(n=17)	(n=9)
Cornified				
Mean	40.4	46.8	13.8	24.6
SDFC#	3**	3***	1**	2*
		4*	2***	
Nucleated				
Mean	3.3	0.6	5.1	0.0
SDFC#	3**	1**	1**	1**
	2**			
	4**			
Leukocytes				
Mean	56.4	52.6	82.1	75.4
SDFC#	3**	3**	1**	2*
		4*	2**	

SDFC# = Significantly different from column number; * = $p < .05$; ** = $p < .01$; *** = $p < .001$; Mont = montane voles; Mead = meadow voles; Pr(S1) = squad I prairie voles; Pr(S2) = squad II prairie voles

Table 3-5. Percentage of Cell Types in Vaginal Smears of Nulliparous Females on Day 2.

	Species (column)			
	1	2	3	4
	Mont	Mead	Pr(S1)	Pr(S2)
	(n=22)	(n=21)	(n=17)	(n=11)
Cornified				
Mean	53.6	50.4	11.6	13.8
SDFC#	3***	3***	1***	1**
	4**	4***	2***	2***
Nucleated				
Mean	1.1	1.0	0.4	0.5
SDFC#				
Leukocytes				
Mean	45.2	47.0	88.0	85.9
STD	34.6	29.2	9.8	13.2
SDFC#	3***	3***	1***	1**
	4**	4***	2***	2***

SDFC# = Significantly different from column number; * = $p < .05$; ** = $p < .01$; *** = $p < .001$; Mont = montane voles; Mead = meadow voles; Pr(S1) = squad I prairie voles; Pr(S2) = squad II prairie voles

Table 3-6. Percentage of Cell Types in Vaginal Smears of Nulliparous Females on Day 3.

	Species (column)			
	1	2	3	4
	Mont	Mead	Pr(S1)	Pr(S2)
	(n=21)	(n=21)	(n=15)	(n=9)
Cornified				
Mean	52.8	47.4	15.7	13.8
SDFC#	3**	3**	1**	1**
	4**	4**	2**	2**
Nucleated				
Mean	0.5	0.6	1.7	0.8
SDFC#				
Leukocytes				
Mean	46.8	52.1	82.5	85.6
SDFC#	3**	3***	1**	1**
	4**	4**	2***	2**

SDFC# = Significantly different from column number; * = $p < .05$; ** = $p < .01$; *** = $p < .001$; Mont = montane voles; Mead = meadow voles; Pr(S1) = squad I prairie voles; Pr(S2) = squad II prairie voles

Table 3-7. Percentage of Cell Types in Vaginal Smears of Parous Females on Day 1.

	Species (column)			
	1	2	3	4
	Mont	Mead	Pr(S1)	Pr(S2)
	(n=10)	(n=13)	(n=10)	(n=9)
Cornified				
Mean	32.7	50.9	14.0	12.1
SDFC#	3*	3**	1*	1*
	4*	4**	2**	2**
Nucleated				
Mean	0.3	0.0	3.4	0.0
SDFC#				
Leukocytes				
Mean	67.0	49.1	82.7	87.9
SDFC#	3*	3**	1*	1*
	4*	4**	2**	2**

SDFC# = Significantly different from column number; * = $p < .05$; ** = $p < .01$; *** = $p < .001$; Mont = montane voles; Mead = meadow voles; Pr(S1) = squad I prairie voles; Pr(S2) = squad II prairie voles

Table 3-8. Percentage of Cell Types in Vaginal Smears of Parous Females on Day 2.

	Species (column)			
	1	2	3	4
	Mont	Mead	Pr(S1)	Pr(S2)
	(n=10)	(n=13)	(n=12)	(n=8)
Cornified				
Mean	33.0	48.0	22.6	13.4
SDFC#		4*		2*
Nucleated				
Mean	1.2	0.4	0.4	0.6
SDFC#				
Leukocytes				
Mean	65.9	51.8	77.0	86.0
SDFC#		4*		2*

SDFC# = Significantly different from column number; * = $p < .05$; ** = $p < .01$; *** = $p < .001$; Mont = montane voles; Mead = meadow voles; Pr(S1) = squad I prairie voles; Pr(S2) = squad II prairie voles

Table 3-9. Percentage of Cell Types in Vaginal Smears of Parous Females on Day 3.

	Species (column)			
	1	2	3	4
	Mont	Mead	Pr(S1)	Pr(S2)
	(n=10)	(n=13)	(n=12)	(n=8)
Cornified				
Mean	40.5	52.0	17.5	18.9
SDFC#	3*	3**	1*	2*
		4*	2**	
Nucleated				
Mean	0.4	0.5	0.3	1.5
SDFC#				
Leukocytes				
Mean	59.0	47.5	82.3	79.8
SDFC#	3*	3**	1*	2*
		4*	2**	

SDFC# = Significantly different from column number; * = $p < .05$; ** = $p < .01$; *** = $p < .001$; Mont = montane voles; Mead = meadow voles; Pr(S1) = squad I prairie voles; Pr(S2) = squad II prairie voles

Table 3-10. Mean Percentage of Cell Types in Smears From Females on the Day of Copulation.

	Mont	Mead	Pr(S1)	Pr(S2)
Nulliparous	(n=14)	(n=2)	(n=8)	(n=3)
Cornified	79.3	61.5	63.3	16.7
Nucleated	0.1*	0.0	4.3*	0.0
Leukocytes	20.6	39.0	32.4	83.3
Parous	(n=8)	(n=8)	(n=9)	(n=4)
Cornified	56.6	56.9	68.8	60.0
Nucleated	0.0	0.1	4.0	1.5
Leukocytes	43.4	43.1	27.3	38.3

* = Mann-Whitney U test ($U = 22.50$, $p < .05$); Mont = montane voles; Mead = meadow voles; Pr(S1) = squad I prairie voles; Pr(S2) = squad II prairie voles

Table 3-11. Percentage of Cornified Cells in Smears of Females That Copulated Versus the Highest Percentage of Cornified Cells in Females That Did Not Copulate.

	Copulators		Non-copulators		
	N	% cornified	N	% cornified	p
Nulliparous					
Mont	14	79.3	8	83.3	ns
Mead	2	61.5	19	64.6	no test
Pr(S1)	8	63.3	15	42.7	ns
Pr(S2)	3	16.7	9	56.2	no test
Parous					
Mont	8	56.6	2	96.0	no test
Mead	8	56.9	5	85.6	ns
Pr(S1)	9	68.8	3	42.3	no test
Pr(S2)	4	60.0	5	66.8	ns

ns = not significant; Mont = montane voles; Mead = meadow voles; Pr(S1) = squad I prairie voles; Pr(S2) = squad II prairie voles

Table 3-12. Mean Percentage of Cornified Cells in Smears on the Day of Copulation Versus the Day Before Copulation.

		Day of cop	Day before cop	
	N	% cornified	% cornified	p
Nulliparous				
Mont	11	81.3	45.7	$p < .01$*
Mead	2	61.5	34.0	no test
Pr(S1)	8	63.3	41.3	ns
Pr(S2)	3	16.7	33.3	no test
Parous				
Mont	3	79.3	44.7	no test
Mead	3	73.3	57.0	no test
Pr(S1)	9	69.1	18.0	$p < .01$*
Pr(S2)	4	60.0	7.8	ns

* = Wilcoxon test; cop = copulation; ns = not significant; Mont = montane voles; Mead = meadow voles; Pr(S1) = squad I prairie voles; Pr(S2) = squad II prairie voles

Table 3-13. Effects of Parity on the Proportions of Females Attaining Behavioral Receptivity.

	Proportion receptive		
	Nulliparous	Parous	T2
Montane			
All females	14/22 = 64%	8/10 = 80%	
A + B females	8/10 = 80%	8/10 = 80%	0
Meadow			
All females	2/21 = 10%	8/13 = 62%	
A + B females	2/13 = 15%	8/13 = 62%	6*
Prairie (S1)			
All females	8/23 = 35%	9/12 = 75%	
A + B females	7/12 = 58%	9/12 = 75%	2
Prairie (S2)			
All females	3/12 = 25%	4/9 = 44%	
A + B females	2/9 = 22%	4/9 = 44%	2
Overall			
All females	27/78 = 35%	29/44 = 66%	
A + B females	19/44 = 43%	29/44 = 66%	10*

* = $p < .05$

Table 3-14. Reproductive Physiology and Social Organization.

Species	Smear type with no male exposure
Prairie voles (*M. ochrogaster*)	Leukocyte predominance (Richmond & Conaway, 1969) (Taylor, this study)
Pine voles (*M. pinetorum*)	Leukocyte predominance (Schadler & Butterstein, 1979)
Montane voles (*M. montanus*)	Cornified predominance (Sawrey, 1989) (Taylor, this study)
Meadow voles (*M. pennsylvanicus*)	Cornified predominance (Clulow & Mallory, 1970) (Taylor, this study)
Field voles (*M. agrestis*)	Cornified predominance (Breed & Clarke, 1970) (Milligan, 1974)
California voles (*M. californicus*)	Cornified predominance (Greenwald, 1956)

Social/asocial* and mating system	Direct male contact needed for receptivity?
Social, monogamous (Getz & Hofmann, 1986)	Yes (> 24hr) (Carter et al. 1987) (Taylor, this study)
Social, monogamous (Fitzgerald & Madison, 1983)	Yes (Schadler & Butterstein, 1979) (Lepri, 1986)
Asocial, polygynous (Jannett, 1980, 1982)	No (Taylor, this study)
Asocial, promiscuous (Madison, 1980a,b)	No (Taylor, this study)
Asocial, polygynous (Myllymaki, 1977)	No (Chitty & Austin, 1957)
Asocial, polygynous (Ostfeld, 1986)	?

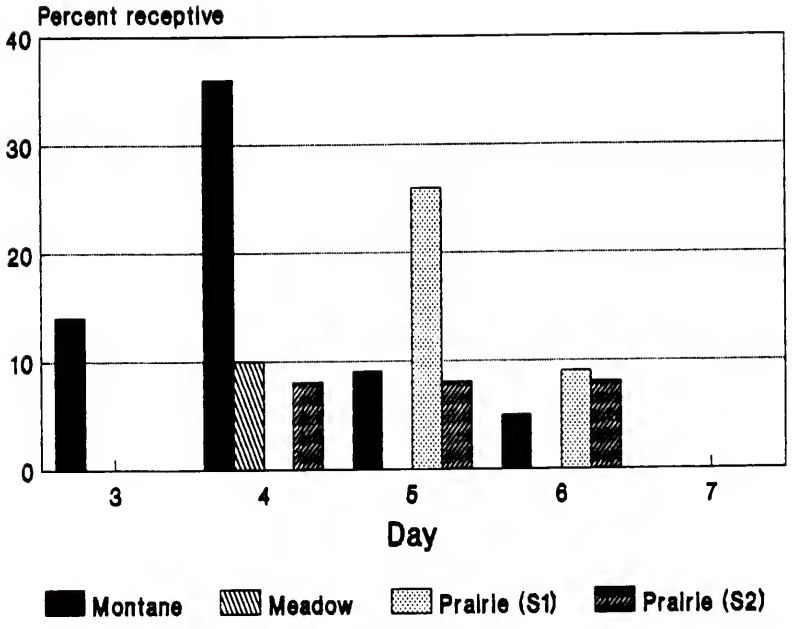

Figure 3-1. Percentage of nulliparous females attaining behavioral receptivity from Day 3 (male first introduced) through Day 7.

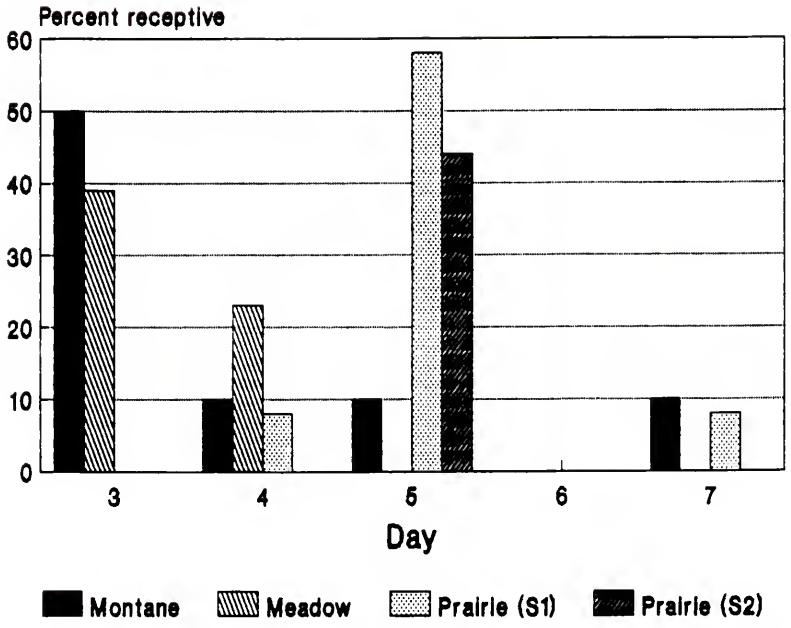

Figure 3-2. Percentage of parous females attaining behavioral receptivity from Day 3 (male first introduced) through Day 7.

CHAPTER 4
PREGNANCY MAINTENANCE IN MONTANE VOLES

Introduction

This chapter is concerned with stud-male mediated maintenance of pregnancy in montane voles. Five experiments are reported.

Pregnancy Maintenance

One possible primer effect that has remained relatively unexplored is stud-male mediated maintenance of pregnancy. There are data to suggest that leaving a male and female together for a time after insemination results in a higher probability of pregnancy than if the male is removed immediately (see Brown, 1985). Ferguson et al. (1987) have noted that the concept of pregnancy maintenance is important at proximate and ultimate levels of explanation. At a proximal level, an understanding of the workings of primer pheromones must include an integration of the major primer phenomena (e.g., pregnancy block, puberty modulation, advancement of ovulation). Attempts at such an integration have been made (e.g., Bronson, 1979a). If pregnancy maintenance proves to be a reliable effect, it must be considered within this integration.

As for ultimate causation, pregnancy maintenance can be viewed in relation to reproductive success from both female

and male perspectives. Females that identify environments ill suited for reproduction and respond by terminating further investment would conserve energy and time, resulting in an increase in lifetime reproductive success (Storey, 1986a; Wynne-Edwards et al., 1987). Ferguson et al. (1987) suggest that stud-male mediated pregnancy maintenance may represent an evolved female pattern that functions to promote reproduction only when males are present to aid in the care of offspring. From the male perspective, Ferguson et al. point out that the pregnancy maintenance phenomenon would affect the costs and benefits of remaining with a mating partner versus seeking additional mates. Thus, the pregnancy maintenance may vary with social organization and mating systems.

Methods of Study

Evidence of pregnancy maintenance can be obtained by leaving males in contact with females for varying amounts of time after mating has begun (e.g., Richmond & Stehn, 1976; Wynne-Edwards et al., 1987). If females that are left in contact with the stud for longer durations are more likely to give birth than females left in contact for shorter durations, then a pregnancy maintenance effect is inferred. One flaw with this method however, is that the effects of male presence and extended copulatory behavior may not be separable. The probability of successful pregnancy increases with increasing amounts of vaginal stimulation (see Dewsbury, 1978). Thus, females left with males for

greater durations may be more likely to give birth due to extended copulation rather than an effect of male presence per se.

The effects of extended copulation can be separated from male presence by confining the male behind a mesh barrier at some predetermined time after mating onset (Berger & Negus, 1982; Ferguson et al., 1987). Doing so prevents additional vaginal stimulation but presumably allows any olfactory, visual, or auditory cues that may be critical for pregnancy maintenance.

Literature Review

It must be emphasized that pregnancy maintenance has received far less formal study than the more well known primer phenomena. Evidence may be found in studies primarily concerned with some other aspect of pregnancy and reproduction. One of the most striking aspects of the literature is the often contradictory nature of different studies. This will be evident in the following literature review. For almost every positive demonstration of a pregnancy maintenance effect, there is a corresponding report of negative results. It would seem enough positive results exist to suggest pregnancy maintenance is a real effect. Further study is warranted to determine the conditions under which this rather elusive phenomenon does and does not occur.

Richmond and Stehn (1976) reported that between 1 and 4 days of cohabitation are necessary for maximum conception

rates in prairie voles. When males were left with females after mating for 4 days, 12 days, or the entire gestation period, conception rates were 95%, 100%, and 97% respectively. When males were removed 24 hr or less after mating, the conception rate was 35%. While these results suggest a pregnancy maintenance effect, firm conclusions cannot be drawn. No information was presented on exactly how soon males in the 24 hr or less group were removed after mating. Prairie voles continue copulatory behavior for extended durations (Pierce et al., 1988), with mounting being observed up to 24 hr under some conditions (Carter et al., 1986), and greater vaginal stimulation has been shown to enhance pregnancy rates (Dewsbury, 1978). Thus, the low pregnancy rate in the 24 hr or less group may be due to reduced amounts of copulatory stimulation, rather than the lack of male presence per se after copulation.

Ferguson et al. (1987) conducted four experiments with prairie voles in which females mated for two ejaculations and then either remained alone or across a mesh barrier from the stud. Prairie voles from two different colonies were used, and the reproductive experience of females (parous vs. nulliparous) and cage changing (remain in cage where mating occurred vs. move to clean cage after mating) were also manipulated. An effect on pregnancy maintenance was only found in females from one colony of prairie voles that were parous and left in the same cage in which mating occurred. Ferguson et al. concluded that pregnancy maintenance can be

demonstrated in prairie voles, but only under a restricted set of conditions.

Dewsbury et al., (1979) observed a doubling of the pregnancy rate if the stud was housed across a mesh barrier in prairie vole females mated for one ejaculatory series in postpartum estrus. This was only a pilot study however, with a small sample size.

Berger and Negus (1982) reported stud-male maintenance of pregnancy in montane voles. Males were allowed to mate for 2 hr following the initial observation of copulation, and then were partitioned from the female by a wire mesh screen which divided the cage in half. A 73% pregnancy rate was observed in females partitioned from a male after 2 hr of mating activity. Significantly fewer females were pregnant (30%) if the male was simply removed after 2 hr of mating, or if females were partitioned to one half of the cage and the male removed after 2 hr of mating (30%).

Other work with montane voles indicates that the pregnancy maintenance effect may be mediated through the olfactory senses. Kranz and Berger (cited as "in preparation" in Richmond & Stehn, 1976) reported that removal of the stud after 2 hr and placing him in a wind tunnel where only his odors reached the female resulted in a significant increase in pregnancy rate. This study has apparently never been published however.

Not all work with montane voles has shown an effect of male presence. Ferguson et al. (1987) used females mated

for two ejaculations. Pregnancy rates were not significantly different in females housed across a mesh barrier from the stud versus females with the stud removed. Moving animals to a clean cage versus leaving animals in the cage were mating occurred was found to significantly reduce pregnancy rates in both conditions.

Wynne-Edwards et al. (1987) observed a complex effect of post-copulatory pair contact on pregnancy rate in virgin female Djungarian hamsters (Phodopus campbelli). Forty-one percent of females littered when allowed 24 hr of post-copulatory contact with their stud male. Females exposed to their studs for either longer or shorter durations (i.e., stud removed immediately after mating, or 48 hr, 96 hr, or 18 days after mating) had significantly higher pregnancy rates. Ferguson et al. (1987) found no evidence of pregnancy maintenance in (P. sungorus campbelli) females mated for two ejaculations in a neutral test arena and then either placed in one half a cage across a mesh barrier from the stud, or placed in the same type of cage with no stud. Pregnancy rates were 90 and 100% respectively.

Norris (1985) investigated a variety of exteroceptive factors including cage changing, vaginal smears, exposure to strange males, and removal of the stud male in a study of pregnancy failure in Mongolian gerbils (Meriones unguiculatus). A significant reduction in pregnancy rate was only observed when the newly mated female was separated

from her stud. This effect was found to occur when the stud was removed the day after mating, but not five days after mating, and did not occur if the female was lactating. However, Rohrbach (1982) reported 100% pregnancy rates in Mongolian gerbil females that were housed across a barrier from the stud after mating, and in females housed alone after mating.

There are also reports of stud males facilitating pregnancy by reducing the incidence of pregnancy block in females exposed to strange males. This has been observed with house mice (Parkes & Bruce, 1961), deer mice (Peromyscus maniculatus) (Terman, 1969), and meadow voles (Storey, 1986b).

In summary, there are a variety of reports in the literature of a pregnancy maintenance phenomenon. However, the effect seems to occur only under a restricted set of conditions. Different laboratories obtain different results even when working with the same species. Clearly, a more detailed analysis of the conditions under which pregnancy maintenance does and does not occur is needed. Without such an analysis, it is difficult to determine how (if) the phenomenon is important in the field.

Plan of Experiments

Montane voles were used as subjects in the following experiments. There are a number of reasons for doing so. Since pregnancy maintenance is a rather elusive phenomenon, it would seem prudent to begin with a species that has, at

least under some conditions, been reported to show the effect (see Berger & Negus, 1982; Ferguson et al., 1987). Montane voles breed well in the laboratory and are thus suited to studies of reproduction. The colony of montane voles used in the following experiments is descended from the population used by Berger and Negus (1982) in which the effect was demonstrated. Finally, work with montane voles may lead to work with other Microtus species that vary in social organization and mating systems. As noted above, the manifestation of pregnancy maintenance may vary with these factors.

Experiments 1 and 2 were designed to be basic demonstrations of the pregnancy maintenance effect. The study of pregnancy maintenance was extended to parous females in Experiment 3. In Experiments 4 and 5 a visitation procedure was developed in an effort to relate the pregnancy maintenance effect to the normal role of the male montane vole in the field. The effects of the stud-male presence versus the effects of extended copulation were also evaluated with respect to pregnancy maintenance.

Experiment 1

Experiment 1 was designed to demonstrate the basic effect of stud-male presence on pregnancy rates in montane voles. If the effect can be demonstrated then further dissection of the conditions under which it occurs will be possible. The basic hypothesis was that female montane voles with the stud present behind a screen will be more

likely to maintain a pregnancy than females without the stud present.

Procedure

Subjects were 38 nulliparous female (90 - 140 days old) and 30 sexually experienced male M. montanus (85 - 240 days old). Animals were tested in 48 x 27 x 13 cm clear polycarbonate divided cages with removable wire mesh partitions. In Chapter 2 further details concerning subjects, housing, and apparatus are available.

Females were taken from their littermate groups and individually housed in one half of a divided cage. Females were tested and housed in the same divided cage for the duration of the experiment, as cage changes have been shown to have a negative influence on pregnancy rates (Clulow et al., 1982; Ferguson et al., 1987). Three days after the placement of the female in the divided cage, a sexually experienced male was placed in the other half (Day 1). After a 15 min habituation period the divider was removed and the animals allowed to interact for 1 hr. The divider was always removed 0.5 - 2 hr into the dark phase of the light-dark cycle. If animals were not observed to copulate within 1 hr the partition was re-inserted. Animals that were observed to copulate were allowed to engage in undisturbed sexual activity for 2 hr. The divider was then replaced to separate the animals. Females were then placed in one of two conditions: (1) the male was removed and the female was left partitioned in one half of the divided cage,

or (2) the male remained across the screen from the female until the birth (or expected birth) of the litter.

To increase the number of receptive females, an estrus induction period was allowed for females that did not copulate on Day 1. Females remained in the divided cages across from the male and were tested for up to three subsequent days (Days 2, 3, and 4), or until they were observed to copulate. During the estrus induction period male odors may build-up on the side of the cage soiled by the male and influence the female even after he is physically removed. Therefore, just before the test period on Days 2, 3, and 4, each male was briefly removed and the soiled bedding on the male's side of the cage was replaced with clean bedding.

Females that did not copulate in the first trial (Days 1 - 4) were retested with a different male in the same manner in a second trial at a later time (usually the following week). Any females that did not copulate in the retesting trial were removed from the study.

Two weeks after copulation was observed a small portion of bedding was removed from each cage and replaced with fresh bedding in order to prevent the build-up of excessively soiled bedding. Starting twenty days after copulation each cage was checked daily for litters.

Results and Discussion

Copulation. Of the 38 females tested, 22 copulated. Sixteen males copulated (some males were used more than

once). Twelve females copulated during the first trial and 10 copulated during the second trial. The number of females mated on each of Days 1 through 4 is presented in Table 4-1.

Pregnancy maintenance. One female in the "male present" condition was found dead the day after the litter was due. This female was removed from the analysis as illness may have altered the likelihood of birth occurring. Females with a male across the barrier were significantly more likely to produce a litter (6 of 11 = 55%) than females without a male present (0 of 10 = 0%, Fisher exact probability test $p = .0085$). Litter sizes ranged from 1 to 5 pups ($M = 2.8$). The number of litters produced by females that copulated on each of Days 1 through 4, and the mean litter sizes for each day are presented in Table 4-1.

Thus, stud-male mediated maintenance of pregnancy was demonstrated like that observed by Berger and Negus (1982). Recall that Ferguson et al. (1987) did not observe pregnancy maintenance in montane voles. However, Ferguson et al. did not control for the build-up of stud odor during the estrus induction period. This procedural difference may have contributed to the discrepancy between their results and the present results.

Experiment 2

Experiment 2 was a direct replication of Experiment 1. Given the array of conflicting results in the literature, and even in this laboratory (i.e., Ferguson et al. vs. Experiment 1 above), it would seem prudent to replicate this

basic demonstration of pregnancy maintenance. Again, the basic hypothesis was that female montane voles with the stud present behind a screen will be more likely to maintain a pregnancy than females without the stud present.

Procedure

Subjects were 58 nulliparous female (80 - 120 days old) and 29 sexually experienced male M. montanus (105 - 300 days old). Procedures were as described in Experiment 1 except that males were not used again if they copulated in a test.

Results and Discussion

Copulation. Twenty-nine of the 58 females tested copulated with 29 different males. Eighteen females copulated during the first trial and 11 copulated during the second trial. One female died before the second trial. The number of females mated on each of Days 1 through 4 is presented in Table 4-1.

Pregnancy maintenance. Three females were found dead before their litters were due, and were removed from the analysis. Females with a male across the barrier were significantly more likely to produce a litter (7 of 13 = 54%) than females without a male present (0 of 13 = 0%, Fisher exact probability test $p = .0026$) Litter sizes ranged from 1 to 4 pups ($M = 2.7$). The number of litters produced by females that copulated on each of Days 1 through 4, and the mean litter sizes for each day are presented in Table 4-1.

Thus, in both Experiments 1 and 2 an effect of stud's presence on the maintenance of pregnancy was observed. Females without a male present after copulation did not maintain pregnancies, whereas approximately 55% of females did if the male remained present behind the barrier after copulation.

Experiment 3

Females with reproductive experience were used in Experiment 3. There are a number of reasons for attempting a demonstration of pregnancy maintenance using fertile animals. First, a practical consideration is the number of females needed for a study. It has been observed that parous females attain estrus more reliably than nulliparous females in experiments on estrus induction (see Chapter 3). Second, there is the benefit of preventing infertile animals from influencing the primary dependent variable. Studies of reproduction in voles (e.g., Kirkpatrick & Valentine, 1970; Negus & Pinter, 1966; Richmond & Conaway, 1969) indicate that a portion of females never successfully breed. Third, parity has been shown to affect similar processes (Clulow et al., 1982; Stehn & Jannett, 1981) with parous females generally being more likely to produce a litter. Ferguson et al. (1987) observed a trend suggesting a parity effect in montane voles.

Although there are potential effects of parity, it was hypothesized that parous female montane voles with the stud

present behind a screen will be more likely to maintain a pregnancy than females without the stud.

Procedure

Subjects were 42 female (125 - 180 days old) and 43 male M. montanus (100 - 270 days old). All animals were known to be fertile. Some fertile males were available from Experiment 1. Additional fertile animals were obtained by pairing males and females in 48 x 27 x 13 cm or 29 x 19 x 13 cm cages for 16 - 20 days. Males were then removed and housed individually in 29 x 19 x 13 cm cages. Litters were taken from females shortly after birth. Females were not tested for at least 10 days (generally longer) after the litter was removed. Pairs that were fertility tested together were not paired in the actual experiment.

The procedures for the actual testing of the animals were the same as Experiment 1 except that males were not used again if they copulated in a test.

Results and Discussion

Copulation. Thirty-five of the 42 females tested copulated with 35 different males. Thirty-two females copulated during the first trial and 3 copulated during the second trial. Five animals did not copulate in two trials and two animals died before their second trial. The number of females mated on each of Days 1 through 4 is presented in Table 4-1.

Pregnancy maintenance. Two females in the "male present" condition were removed from the analysis as the

males across the barrier died before the litter was due. Females with a male across the barrier had a slightly higher pregnancy rate (7 of 15 = 47%) than females without a male present (7 of 18 = 39%), but this difference was not significant (X^2 [1, N = 33] = 0.20, ns). Litter sizes ranged from 2 to 8 pups ($\underline{M}$ = 4.4). The number of litters produced by females that copulated on each of Days 1 through 4, and the mean litter sizes for each day are presented in Table 4-1. Mean litter size did not differ significantly by condition ($\underline{M}$ = 5.14 "male absent" condition vs. $\underline{M}$ = 3.7 "male present" condition, $\underline{t}$ (12) = 1.87, n.s., see Table 4-2).

Early each year a breeding depression is observed in the montane vole colony. This breeding depression also apparently occurred in the animals used during this time period in this experiment. Of 10 females in which copulation occurred in January, only one ("male absent" condition) produced a litter. If these 10 animals are removed from the primary analysis (5 "male present", 5 "male absent"), again pregnancy maintenance is not observed. Females with a male across the barrier did have a higher pregnancy rate (7 of 10 = 70%) than females without a male present (6 of 13 = 46%), but this difference is not significant (X^2 [1, N = 23] = 1.31, ns).

Thus, in contrast to nulliparous females (Experiments 1 and 2), an effect of stud-male presence on pregnancy was not observed in parous females.

Experiment 4

The above experiments seem to indicate that while pregnancy maintenance is a reproducible phenomenon, it seems to occur only under certain conditions. To further investigate pregnancy maintenance, one might try to design experiments in light of what is known about the behavior of montane voles in the field. Experiments with greater ecological relevance may shed some light on how (if) pregnancy maintenance operates in the field.

One consideration might be the normal role of the male in the field. Pregnancy maintenance has been demonstrated in prairie voles (Ferguson et al., 1987; Richmond & Stehn, 1976) and in the field a male and female often form a pairbond and share the same nest site (Getz & Hofmann, 1986; Getz et al., 1981). Thus a male-mediated enhancement of the probability of pregnancy seems to mesh well with the normal behavior of prairie voles in the field. Partitioning studs from females (e.g., Ferguson et al., 1987) seems an appropriate procedure for study as males apparently spend much time with the female.

Male and female montane voles apparently do not form a pairbond and/or share the same nest site (Jannett, 1980, 1982). Rather, male montane voles defend territories that overlap the territories of one or more females. They may concentrate daily activity in the immediate vicinity of the female or females within the territory, perhaps foraging together (Jannett, 1982). Thus, the laboratory procedure

where the stud is left across the screen for 24 hr a day is rather unrealistic with respect to behavior that occurs in the field. A more appropriate procedure might involve daily "visits" by the stud to the female. Thus, the efficacy of daily visits in the maintenance of pregnancy might be assessed.

Another issue that can be addressed experimentally is the separation of the effects of male presence per se from extended durations of copulatory behavior. Montane voles are induced ovulators with an induced luteal phase. Limited mating may result in ovulation, but the resulting corpora lutea degenerate rapidly (Kenney & Dewsbury, 1977). Berger and Negus (1982) report that continued mating activity may be an important component of pregnancy maintenance. They found that significantly fewer females maintained their pregnancies when partitioned from males rather than allowed continuous access, and suggest that repeated mating in the continuous contact condition reinforces the pregnancy maintaining neuroendocrine environment.

An alternative interpretation however, is that the presence of the stud odor may facilitate the maintenance of the corpora lutea and implantation (Brown, 1985). In other words, after a given amount of mating, further enhancement of the probability of pregnancy may arise in part or fully from an aspect of male presence (e.g., odor) other than additional copulatory stimulation. Data reported by Berger and Negus (1982) show that in conditions where the stud

remained in contact with the female for 24 hr or more (either partitioned after 2 hr of copulation, or allowed continuous contact) pregnancy rates were high (66% or greater). When the stud was removed after 2 hr, pregnancy rates were significantly lower (30%).

Odors present at the time of mating are thought to be important in processes related to pregnancy maintenance. Keverne and de la Riva (1982) have described "neuroendocrine memory" system in female house mice that prevents the stud from blocking a pregnancy for which he is responsible. The odor cues associated with mating are identified as what the female's neuroendocrine system "recognizes", allowing the distinction between stud and strange males.

Prolonged copulation has been observed in Microtus; perhaps one function of prolonged copulation is to allow male odors to influence the female neuroendocrine system. Prairie voles continue copulatory behavior for extended durations (Pierce et al., 1988), with mounting observed up to 24 hr after the initiation of mating under some conditions (Carter et al., 1986). Berger and Negus (1982) report anecdotal observations of mating activity occasionally up to 48 hr following the initiation of copulation in montane voles. Thus, when a male is removed from a female after, for example, 2 hr of mating, reductions in pregnancy may be due to loss of additional copulatory stimulation, loss of male odor, or both. By using cages divided with screens it will be possible to control duration

of both copulatory stimulation and exposure to other male-related stimuli around the time of mating.

Thus, Experiment 4 was designed to address two issues. First, the efficacy of male "visits" of a set duration in maintaining pregnancy will be investigated. Doing so may provide information on how and if pregnancy maintenance occurs in the field. Second, work to date with montane voles (Berger & Negus, 1982; Ferguson et al., 1987) has not clearly separated the effects of extended durations of copulatory behavior versus equal durations of male presence with reduced copulatory stimulation. It may be that both extended durations of copulatory behavior (see Dewsbury, 1978) and the duration of exposure to male odor immediately after mating both contribute to the enhancement of the probability of pregnancy.

Procedure

Subjects were 87 female (90 - 150 days old) and 85 male M. montanus (100 - 330 days old). The basic methods (testing conditions etc.) were the same as Experiment 1, except that females were run in three four-day trials before being removed from the experiment for not copulating, and males were not used again if they copulated in a test.

When mating behavior was observed, females were placed in one of the five following conditions:

Group 1: Females received 2 hr of uninterrupted mating, after which the stud was removed and the female partitioned to one half of the divided cage. Starting on

the following day, the stud was returned to the divided cage across the screen from the female each day for 12 consecutive days. Each "visit" began at 1145 hr (+ or - 15 min) and was 4 hr in duration.

Group 2: Females received 2 hr of mating, after which the male was removed and placed in a separate cage. The female remained partitioned in one half of the divided cage.

Group 3: Pairs were left undisturbed for 24 hr after the initiation of mating, after which the male was removed and the female partitioned to one half of the cage.

Group 4: Females received 2 hr of mating after which the cage divider was re-inserted. The stud remained across from the female for 22 hr. The stud was then removed from the divided cage after the 22 hr exposure period (24 hr after the onset of mating).

Group 5: Females received 2 hr of mating followed by 22 hr of exposure to the stud partitioned behind a screen. Starting the next day the stud was returned to the divided cage across the screen from the female for 11 consecutive days. "Visits" were identical in timing and duration to those received by animals in Group 1.

Results and Discussion

Copulation. Sixty-two of the 87 females tested copulated with 62 different males. Twenty-nine females copulated on the first trial, 25 on the second trial, and 7 during the third trial. The number of females mated on each of Days 1 through 4 are presented in Table 4-1.

Pregnancy maintenance. One female died before the litter was due and was removed from the analysis. No significant differences were found among the groups in pregnancy rate (X^2 (4, N = 61] = 3.21, ns) (see Table 4-3).

Further analyses were conducted. When Groups 1 and 5 were combined (receiving daily visits: 33% pregnant) and compared to Group 2 (2 hr mating: 15% pregnant) the difference was not significant (Fisher exact probability test p = .2191). When Groups 1, 3, 4, and 5 were combined (each having male exposure beyond 2 hr: 25% pregnant) and compared to Group 2 (2 hr mating: 15% pregnant) the difference was not significant (Fisher exact probability test p = .3742). Mean litter sizes by condition are presented in Table 4-2.

In brief, the objectives of Experiment 4 were to 1) determine if visits by the stud can maintain pregnancies, and 2) attempt to separate the effects of extended copulation from male presence without copulation. The results indicate that daily visits of the duration used are ineffective in increasing the pregnancy rate compared to females which received only 2 hr of copulation. While pregnancy rates were higher in the groups receiving visits, the differences were not significant.

Few inferences can be made regarding the effects of extended copulation versus equal duration of male presence without copulatory behavior. Neither the group with 24 hr unrestricted contact, or the group with 2 hr mating and 22

hr exposure across a screen differed significantly in pregnancy rate from the group receiving 2 hr mating only. Contrary to Berger and Negus's (1982) suggestion, it appears that extended durations of copulation beyond 2 hr does not contribute to an enhanced pregnancy rate, at least under these conditions.

Experiment 5

Although there was no evidence of a significant pregnancy maintenance effect in Experiment 4, there did appear to be a trend toward higher pregnancy rates in groups with greater male exposure (see Table 4-3). Experiment 5 was designed to follow-up this trend.

The likelihood of observing a pregnancy maintenance effect may be enhanced by increasing the duration of each daily visit. One might argue that to do so seems inconsistent with the evidence showing montane voles do not cohabit in the field (Jannett, 1980, 1982). In effect, how long can a "visit" be before it becomes "co-habitation". On the other hand, while a divided cage provides some contact, it still does not allow the degree of contact freely interacting animals have. Thus, a "visit" duration longer than that which occurs in the field may be necessary to compensate.

Pregnancy maintenance might also be promoted by increasing the number of days that the female is exposed to the stud. Microtus are unusual in that post-implantation pregnancy disruption occurs (see Stehn & Richmond, 1975;

Kenney et al., 1977). Perhaps Microtus females need daily exposure to male odors over the entire gestation period, and increasing the number of days the stud is exposed to the female may increase the likelihood of an effect of pregnancy maintenance.

Certain experimental procedures were modified to make the compared groups more equivalent. Handling procedures may have contributed to the lack of an effect in Experiment 4. In Experiment 4, Group 2 (2 hr mating) animals were left undisturbed for the entire gestation period. Animals in Groups 1 and 5, which received daily visits, encountered considerable disturbance however, due to the procedures involved in introducing and removing the stud on a daily basis. There is evidence that disturbance can reduce pregnancy rates in Microtus (Ferguson et al., 1987; Stehn & Jannett, 1981). Thus, a more appropriate comparison might involve two groups of animals equated on general disturbance, with one receiving daily visits.

Procedure

Subjects were 44 female (100 - 150 days old) and 46 male M. montanus (90 - 265 days old). The basic methods (testing conditions etc.) were the same as Experiment 1, except that females were run in three four-day trials before being removed from the experiment for not copulating.

When mating behavior was observed, animals were assigned to one of the following two conditions:

Groups 1: After 2 hr of uninterrupted mating behavior, the screen was re-inserted to separate the male and female. The male remained across from the female for the next 22 hr, and was then removed to a separate cage. On each subsequent day for the remainder of the gestation period the male was placed back in the cage across the screen from the female for 8 hr. These 8 hr "visits" began at 1100 hr (+ or - 15 min).

Group 2: After 2 hr of uninterrupted mating behavior the male was removed to a separate cage and the female partitioned to one half of the divided cage. Each day the cages were disturbed (opening, closing, sliding on shelves, etc.) in exactly the same manner as the cages used for animals in Group 1.

Results and Discussion

Copulation. Twenty-nine of the 44 females tested copulated with 29 different males. Twenty-three copulated on the first trial, 5 copulated on the second trial, and 1 animal copulated on the third trial. The number of females mated on each of Days 1 through 4 is presented in Table 4-1.

Pregnancy maintenance. Females that received 8 hr daily visits for the entire gestation period had a higher birth rate than females that did not receive visits (8 of 15 = 53% vs. 3 of 14 = 21%). This difference approached significance (X^2 [1, N = 29] = 3.13, p = .0731). Litter sizes ranged from 1 to 6 pups (M = 3.5). The number of females that copulated on each of Days 1 through 4, and the

mean litter sizes for each day are presented in Table 4-1. Mean litter size by condition is presented in Table 4-2.

Increasing the duration of daily visits did not result in the display of a pregnancy maintenance effect. However, there does appear to be a continuation of the trend of higher pregnancy rates occurring in association with greater duration of post-copulation male exposure (see Table 4-4).

General Discussion

In Experiments 1 and 2 pregnancy maintenance was demonstrated and the effect replicated. The presence of the stud behind a wire mesh barrier after copulation resulted in a significant facilitation of pregnancy in nulliparous females. No effect of the presence of the stud was apparent in Experiment 3 with parous females. In Experiments 4 and 5 the duration of male presence was titrated through a "visits" procedure. Although none of the visit durations resulted in a significant facilitation of pregnancy, there did appear to be a trend with greater durations of male presence after copulation resulting in higher pregnancy rates.

Pregnancy Maintenance: A Real Effect?

As noted in the Introduction, a striking aspect of the pregnancy maintenance literature is the often contradictory nature of the findings. For every positive demonstration of pregnancy maintenance, there seems to be another source of evidence for the same species that indicates no effect. The present results would seem to indicate that pregnancy

maintenance is a real and reproducible phenomenon. The effect was demonstrated in Experiment 1 and replicated with nearly identical results in Experiment 2. Additionally, the present results are quite consistent with work in montane voles reported by Berger and Negus (1982). In both studies an approximately 50 - 55% difference in pregnancy rates was observed between conditions where the male was removed after 2 hr of copulation versus remaining present behind a wire-mesh screen after 2 hr of copulation. Finally, although none of the visitation procedures attempted in the present work produced a significant effect of pregnancy maintenance, there did appear to be a trend of increasing pregnancy rates with greater durations of male exposure after copulation. This is consistent with the notion of a male role in the facilitation of pregnancy after copulation.

The present results may provide some insights regarding the negative results of Ferguson et al. (1987) with montane voles. There are several procedural differences that may be important. Ferguson et al. did not control for the male odor build-up that may have occurred during the estrus induction period. Also, a repeated measures design was used, which resulted in the inclusion of parous females. Parous females did not show an effect of pregnancy maintenance in the present work. Finally, Ferguson et al. used montane voles from a different colony previously maintained at the University of Florida. As this colony is

no longer maintained, the potential effects of colony differences cannot be evaluated.

Effects of Parity

Parity appeared to have a major influence on pregnancy maintenance. Parous females did not show significantly higher pregnancy rates when housed across from the stud after copulation. This finding is consistent with other work indicating that parous females are more likely to become pregnant than nulliparous females. Ferguson et al. (1987) found that parous montane voles generally had higher pregnancy rates than nulliparous females. In studies of male-induced pregnancy disruption, parity was found to reduce disruption rates in prairie and montane voles (Stehn & Jannett, 1981) red-backed voles, Clethrionomys gapperi (Clulow et al., 1981), and deermice (Peromyscus maniculatus bairdii) (Terman, 1969).

In contrast, Ferguson et al. (1987) found that parous, but not nulliparous, prairie vole females of one colony showed a facilitation of pregnancy when housed across from the stud. Prairie voles from a different colony in the Ferguson et al. study showed no effect of pregnancy maintenance however, regardless of parity.

Parity may alter the amount of copulatory stimulation required for pregnancy initiation (Huck & Lisk, 1985; Milligan, 1975), and may thus influence pregnancy maintenance. In the present studies parous females may have had a lower threshold for pregnancy initiation than

nulliparous females, and thus required no post-copulatory male contact for further stimulation. Nulliparous females may need the presence of the male after copulation to reach the pregnancy initiation threshold, perhaps due to some aspect of reproductive immaturity. Bronson (1979a) notes that the presence of a male is essential for the normal organization of puberty in house mice. Perhaps there is a similar situation in montane voles, with the male continuing to stimulate the maturation of the nulliparous female reproductive system after copulation. Thus, the birth of a litter may be the proper indication of reproductive maturation in voles, rather than first estrus, a measure most commonly used (see Vandenbergh, 1983).

An alternative possibility is that females become more sensitive to the presence of the stud after parity. In the present experiments the "male absent" condition consisted of removing the male after copulation and placing him in a different cage in the same room. Perhaps removing the male completely to a different room, to remove all possibility of olfactory or other cues, would reduce the pregnancy rate in the "male absent" condition. This possibility has not been tested.

Effects of Visits

Of the species reported to show the pregnancy maintenance effect, the strongest evidence seems to exist for montane voles. It has been demonstrated in two different laboratories (Berger & Negus, 1982; this study).

The present work may also help explain previous negative findings in montane voles (see Pregnancy Maintenance: A Real Effect? above). That montane voles show the effect is something of a puzzle, in that unlike prairie voles and Mongolian gerbils (e.g., Agren et al., 1989; Getz & Hofmann, 1986), there is little evidence of co-nesting or pairbonding (Jannett, 1980, 1982). Thus, the existence of the pregnancy maintenance effect does not seem to correlate in a straight-forward manner with the normal role of the male in the field. Since male territories do overlap with females and they do spend time together (perhaps foraging together) (Jannett, 1982), the visitation method (Experiments 4 and 5) was developed in an attempt to relate the pregnancy maintenance effect in montane voles to the normal role of the male in the field.

In Experiments 4 and 5, post-mating visits consisted of the stud being present across the wire-mesh barrier from the female. Visit durations used included; 1) the male present for 22 hr immediately after mating, 2) the male present for 4 hr per day for 12 consecutive days after mating, 3) the male present for 22 hr after mating and for 4 hr per day for 11 consecutive days, and 4) the male present for 22 hr after mating and 8 hr per day for the entire gestation period. While none of these conditions resulted in a significantly higher pregnancy rate than the relevant control group, there did seem to be a clear trend toward greater pregnancy rate with greater male exposure (see Table 4-4). The greatest

duration of post-mating male exposure resulted in a difference in pregnancy rates that approached significance ($p = .0731$; see results of Experiment 5). While not conclusive, these data suggest that males may facilitate pregnancy in the field by visiting females during the gestation period.

Further study in this area may take two directions. Montane vole females could be provided with longer visits to determine the sufficient duration for a pregnancy maintenance effect. Additionally, the variability in social organization and mating systems among *Microtus* species should be utilized. Species that do show pairbonding and co-nesting (e.g., pine voles and prairie voles) might be studied with similar methods involving visits. The influence of visits may differ in these species, as the male is normally associated very closely with the female in the field.

Male Presence Versus Extended Copulation

Berger and Negus (1982) suggested that an important component of pregnancy maintenance may be extended durations of copulatory behavior. Limited mating in montane voles produces corpora lutea that degenerate rapidly (Kenney & Dewsbury, 1977). An alternative possibility however, is that the stud odor after mating may facilitate the maintenance of the corpora lutea and implantation (see Brown, 1985). Thus, after a given amount of mating, further enhancement of pregnancy may arise in part or fully from an

aspect of male presence (e.g., odor) other than additional copulatory stimulation.

An attempt to compare these possibilities was made in Experiment 4. Females that were allowed extended durations of copulatory behavior (24 hr full contact with a male) displayed a 20% pregnancy rate. Females allowed only 2 hr of copulation, but were exposed to the stud for 22 hr immediately after mating also displayed a 20% pregnancy rate. However, these rates did not differ significantly from females who only mated with male for 2 hr and received no further exposure. Thus, it appears that neither extended copulation alone, nor limited mating with an equal period of extended male presence is effective in enhancing pregnancy.

Perhaps a more powerful method to detect differences in the effects of extended copulation versus male presence after limited copulation would involve hormone assays. A number of studies have shown that brief exposure of one sex to chemical stimuli from the other sex can have measurable consequences on hormone levels (Dluzen, et al., 1981; see also Bronson, 1979a). For example, it would be interesting to compare progesterone levels during the gestation period in females that receive 2 hr of mating only, females that receive 2 hr mating and the male is present across a screen for the entire gestation period, and females that are allowed ad lib copulation and remain together for the entire gestation period.

The Bruce Effect and Pregnancy Maintenance

It is possible to compare pregnancy failure due to exposure to a strange male (the Bruce effect) and pregnancy failure due to the lack of the stud male (pregnancy maintenance) at proximal and ultimate levels. At a proximal level there are a number of similarities in the manifestation of both effects. There is evidence that both effects occur in greater magnitude in nulliparous females, with parous females being more likely to maintain the pregnancy (Clulow et al., 1982; Stehn & Jannett, 1981; Storey, 1986b; Experiments 1, 2, and 3 of the present work). The physiological mechanisms may be similar. Norris (1985), working with Mongolian gerbils, noted that the pregnancy maintenance was

> restricted to the immediate period after mating. . .; was annulled by concurrent lactation. . .; and was associated with CL failure, embryo degeneration and irregular transport. . . . These events are strikingly similar to those induced by 'strange' male urinary pheromones via olfaction in the mouse, thereby implying a neuroendocrine response to a specific stimulus resulting in cessation of luteotrophin (prolactin implicated) release from the anterior pituitary, followed by luteal failure, progesterone deficiency, and pregnancy block. (page 46)

There is also a potential for considerable overlap in the explanations of pregnancy maintenance and the Bruce effect at an ultimate level. The most convincing adaptive explanations for the Bruce effect involve benefits that accrue to the female (see Labov, 1981; Schwagmeyer, 1979).

Storey (1986b), in discussing the Bruce effect, notes that the ability to reabsorb pregnancies may be especially important for young dispersing females as they can become rapidly accepted to new territories by being receptive to the resident male. This is less of a consideration to older multiparous females that have attained stable territories. This hypothesis could also encompass the pregnancy maintenance phenomenon.

Females that identify environments ill suited for reproduction can potentially save time and energy by reabsorbing embryos that have little chance for survival (Labov, 1981; Story, 1986a; Wynne-Edwards, et al., 1987). Environments ill suited for reproduction may include a lack of a male to help with care of the offspring, or the presence of infanticidal males. The Bruce effect has been proposed as an adaptive mechanism for coping with such situations (Labov, 1981; Schwagmeyer, 1979). Similarly, Ferguson et al. (1987) proposed that pregnancy maintenance may function to promote reproduction only when males are present to assist in the care of the offspring. Pregnancy maintenance may also function in an infanticidal prevention role, as the disappearance of the stud is likely to followed by his replacement by another male. Replacement males may be infanticidal. Such explanations depend on male care and/or infanticide occurring in natural populations. These adaptive explanations have been criticized for relying on

conditions unlikely to occur in natural populations (Bronson & Coquelin, 1980).

Perhaps pregnancy maintenance can be more parsimoniously explained as part of a female reproductive system that is exquisitely tuned to environmental indicators of the probability of future reproductive success. Microtus must reproduce under conditions of great environmental uncertainty. It is not surprizing then, that their reproductive parameters are flexible, efficient, and highly responsive to environmental information (see Negus & Berger, 1987). Female Microtus are particularly dependent on male stimulation for many aspects of reproduction, including puberty acceleration, estrus induction, and ovulation (see Richmond & Stehn, 1975; Sawrey, 1989; Sawrey & Dewsbury, 1985; Chapter 3). It is energetically efficient for females to be reproductively quiescent unless conditions are appropriate for reproduction (see Bronson, 1979a). The extended presence of a male may be an environmental indicator of appropriate conditions for reproduction. In nulliparous female montane voles males can accelerate puberty, induce estrus, induce ovulation through copulation, and maintain pregnancy after copulation. At each step in the system, the loss of the male may result in reproductive failure, thus conserving energy for reproduction at a more appropriate time. Pregnancy maintenance may thus be a part of the complex dependence females have for stimuli from males in Microtus that insures reproduction occurs only

under appropriate conditions. Pregnancy maintenance can therefore be viewed as a functional part of a larger pheromonal cueing system.

Summary and Conclusions

In the present work the rather elusive phenomenon of stud-male mediated maintenance of pregnancy was demonstrated and replicated. These results would seem to indicate that pregnancy maintenance is a real effect that should be integrated with other more well known primer phenomena. It was determined that pregnancy maintenance is only manifested in nulliparous females. This finding, and other procedural differences may account for previous negative findings in montane voles. In order to relate the pregnancy maintenance phenomenon to the normal role of the montane vole male in the field, a visitation procedure was developed. While none of the male visit durations used resulted in a significant pregnancy maintenance effect, there was a clear trend of higher pregnancy rates being associated with greater durations of male presence. It was also shown that neither extended copulation, nor limited copulation with equal duration of male exposure, was effective alone in producing pregnancy maintenance. Pregnancy maintenance may be similar to the Bruce effect in terms of proximal mechanisms. Pregnancy maintenance also resembles the Bruce effect in terms of ultimate explanations, as many an adaptive tale can be readily spun. Pregnancy maintenance may be part of the complex dependence female Microtus show for stimuli from

males, resulting in energetic efficiency in reproduction. Further study in species that show contrasting social organization and mating systems may shed more light on the functional role of pregnancy maintenance.

Table 4-1. Number of Females Mating, Number of Litters, and Mean Litter Size Per Day in Experiments 1 through 5.

Experiment	Measure	Day 1	2	3	4	Total
1	Number mating	3	7	9	3	22
	Number litters	1	1	3	1	6
	Mean litter size	2.0	1.0	3.0	5.0	
2	Number mating	9	8	9	3	29
	Number litters	3	1	1	2	7
	Mean litter size	3.0	4.0	1.0	2.5	
3	Number mating	18	9	6	2	35
	Number litters	8	4	2	0	14
	Mean litter size	4.5	4.3	4.5	0.0	
4	Number mating	12	22	24	3	61
	Number litters	3	4	6	1	14
	Mean litter size	3.7	3.5	3.8	4.0	
5	Number mating	10	5	13	1	29
	Number litters	5	2	4	0	11
	Mean litter size	3.2	3.5	4.0	0.0	

Table 4-2. Litter Size by Condition in Experiments 3, 4, and 5.

Experiment		Condition				
		MP	MA			
3	Number litters	7	7			
	Mean litter size	3.7	5.14			
		Condition (Group*)				
		1	2	3	4	5
4	Number litters	3	2	2	2	5
	Mean litter size	4.0	2.5	5.5	3.0	3.6
		Condition				
		MP	MA			
5	Number litters	8	3			
	Mean litter size	3.6	2.1			

MP = male present after copulation; MA = male absent after copulation; * = see text for Group description

Table 4-3. Results of Experiment 4.

Group	Male exposure	N	Number of litters	Percent pregnant
1	2hr cop/4hr visit	12	3	25%
2	2hr cop	13	2	15%
3	24hr cop	12	2	20%
4	2hr cop/22hr exposure	12	2	20%
5	2hr cop/22hr exposure/ 4hr visit	12	5	42%

cop = copulation

Table 4-4. Observed Pregnancy Rate With Different Durations of Male Exposure After Copulation.

Experiment	Male Exposure	N	Number of litters	Percent pregnant
1	2hr cop	10	0	0
2	2hr cop	13	0	0
4	2hr cop	13	2	15
5	2hr cop*	14	3	21
4	2hr cop/22hr exposure	12	2	20
4	24hr cop	12	2	20
4	2hr cop/4hr visit/ 12 days	12	3	25
4	2hr cop/22hr exposure/ 4hr visit/11 days	12	5	42
5	2hr cop/22hr exposure/ 8hr visit/gestation	15	8	53
1	2hr cop/24hr exposure/ gestation	11	6	55
2	2hr cop/24hr exposure/ gestation	13	7	54

cop = copulation; cop* = disturbance control

CHAPTER 5
GENERAL DISCUSSION

In this final chapter the studies of male-induced estrus (Chapter 3) and stud-male mediated maintenance of pregnancy (Chapter 4) are placed in a larger context of general issues in the study of primer pheromones and their role in reproduction. First, a brief summary of the experimental results of this dissertation is presented. Second, pheromonal cueing systems elucidated through laboratory study are compared in house mice (Mus) and voles (Microtus). Similarities and differences between the genera in estrus induction and pregnancy maintenance, and other primer phenomena, are discussed. Third, drawing on the house mouse model, the role of priming pheromones in the reproductive strategies of voles under natural conditions is discussed. In particular the role of male chemosignals in successful reproduction of Microtus is emphasized. Finally, the value of comparative work identifying species differences in the responsiveness to primer pheromones is discussed. These differences may offer valuable insights into the role of priming pheromones in reproduction in natural populations. Throughout these discussions deficiencies in our knowledge of primer actions in Microtus

are pointed out, and suggestions for future research are made.

Summary of Experimental Results

An investigation of male-induced estrus in nulliparous and parous montane, meadow, and prairie voles was reported in Chapter 3. Nulliparous montane voles copulated with a shorter duration of male exposure than nulliparous prairie voles. Parous montane and meadow voles did not differ in the duration of male exposure needed for receptivity, and copulated with less exposure than parous prairie voles. Montane and meadow voles were observed to copulate without prior direct exposure to males; prairie voles were never observed to do so. In nulliparous females, but not parous females, species differences were found in the proportions of females attaining behavioral receptivity. In general parous females had higher behavioral receptivity rates than nulliparous females.

Species differences were also found in vaginal cytology. Before being exposed directly to males, montane and meadow vole females did not differ in the proportion of cell types, and had greater proportions of cornified cells and smaller proportions of leukocytes in vaginal smears than prairie voles. This was true of both nulliparous and parous females. However, smears taken on the day of copulation from animals of each species tended to be similar; that is, species differences in the proportions of cell types tended to disappear after male exposure leading to copulation. The

evidence of vaginal smears being predictive of behavioral receptivity was mixed. Females of all species that copulated tended to have high levels of cornification, but it was also apparent that high levels of vaginal cornification could occur in the absence of behavioral receptivity.

This evidence of species differences in female reproductive physiology (i.e., vaginal cytology and the duration of male exposure needed for receptivity) was discussed in relation to species differences among Microtus in social organization and mating systems.

An investigation of stud-male mediated maintenance of pregnancy in montane voles was reported in Chapter 4. The presence of the stud-male after copulation increases the likelihood that a nulliparous female montane vole will carry a litter to term. This effect was not observed in parous females. Male post-copulatory presence effected through "visits" appeared to increase the likelihood of the pregnancy being maintained proportional to the amount of post-copulatory male exposure. Neither the opportunity for extended duration of copulation (up to 24 hr), nor limited mating with an equal period of extended male presence, was effective in enhancing pregnancy in nulliparous females. For the probability of pregnancy to be enhanced the male must be present longer than the initial 24 hr after the initiation of copulation. These results were discussed in

terms of the role of male chemosignals in successful reproduction.

Priming Pheromones in House Mice and Voles

It is possible to compare the responses to priming pheromones (i.e., the pheromonal cueing system) in house mice (Mus) and voles (Microtus). Doing so may help conceptualize and integrate the research that has been conducted, and direct future research in a logical and efficient manner. It appears that some primer responses are similar in voles and mice, some differ in degree, and some appear to be fundamentally different. Some primer effects have not received sufficient investigation in voles to make good comparisons.

Puberty acceleration. One of the well known primer effects that appears to be similar in house mice and voles is male stimulated acceleration of puberty in juvenile females. In mice, an androgen dependent urinary cue released from males is responsible for puberty acceleration (see Vandenbergh, 1983). The degree of acceleration is increased when chemical and tactile cues are combined (Drickamer, 1974; Bronson & Maruniak, 1975). The critical hormonal responses involved in the induction of puberty consist of an immediate increase in LH (within 1 - 3 hr of male exposure), followed by a dramatic increase in serum estradiol (Bronson, 1979a).

Puberty acceleration has been demonstrated in several Microtus species, including montane voles (Sawrey, 1989),

meadow voles (Baddaloo & Clulow, 1981), pine voles (Lepri & Vandenbergh, 1986), and prairie voles (Hasler & Nalbandov, 1974). Male urine contains a substance that stimulates reproductive activation (Baddaloo & Clulow, 1981; Carter et al., 1980), and direct contact appears important to produce the maximal effect (Carter et al., 1980; Sawrey, 1989). Interestingly, castrate male urine does produce some reproductive activation in prairie voles, as females exposed to castrate male urine show significant uterine weight increases relative to water exposed controls. However, exposure to intact male urine results in a significant increase beyond castrate male urine (Carter et al., 1980).

Induction of estrus. House mice and voles appear to differ in primer action at the time of estrus in adult females. Adult house mouse females will attain estrus without exposure to males, but have shorter estrous cycles and attain estrus more rapidly when exposed to males than when housed alone. Maximal effects are observed when physical contact is possible, but odors alone have some effect (Brown, 1985; Whitten, 1956). The potency of the male's urine is dependent on androgen, and it appears to stimulate LH release (and subsequent elevation of estrogen) in female recipients (Bronson, 1979a).

Voles are reflex ovulators lacking a spontaneous estrous cycle. Whereas isolated adult female house mice will attain estrus without male exposure, at least some species of Microtus appear to be entirely dependent on

contact with males for the induction of estrus (see Sawrey & Dewsbury, 1985; Chapter 3). As demonstrated in Chapter 3, species may show important differences in the degree of dependence on male contact. The estrus induction mechanism has been delineated for prairie voles, a species that is highly dependent on male contact. Stimuli from males are received by the vomeronasal organ and the main olfactory system (Lepri & Wysocki, 1987). This stimulation induces neuroendocrine reflexes resulting in rapid increases in serum LH levels (see Dluzen et al., 1981), with subsequent elevations in levels of serum estrogen (Cohen-Parsons & Carter, 1987). It is not known how the mechanism may differ in species that are less dependent on male contact. Perhaps the receptors are more sensitive and thus require less direct male contact, or females are physiologically "closer" to estrus (e.g., higher serum estrogen levels).

Primer actions during pregnancy. House mice and voles also differ in primer actions during pregnancy. House mice are susceptible to a strange male pregnancy block commonly known as the Bruce effect. Maximal effects occur prior to implantation (within four days). Contact with urine or soiled bedding is as effective as physical interaction with a strange male. The blocking pheromone is present in male urine and is androgen dependent. The pregnancy block does not occur in lactating females (see Brown, 1985; Milligan, 1980; Parkes & Bruce, 1961).

In general, voles may be susceptible to the Bruce effect for a longer period of time during gestation than house mice. Pregnancy block can occur in prairie voles up to day 17 of gestation (Stehn & Richmond, 1975), and has been demonstrated at day 12 in meadow voles (Storey, 1986b). The post-implantation block may result from a different endocrine mechanism than that responsible for the pre-implantation block of house mice (Milligan, 1980). Other findings indicate contrasts in the manifestation of the Bruce effect within Microtus, as well as providing further contrasts with house mice. For example, Milligan (1976) found that direct, unrestricted contact with intact males was necessary for pregnancy block in field voles (Milligan, 1976); castrate males, and urine and soiled bedding from intact males were ineffective. Smale (1988) found urine alone, from both intact and castrate males, effective in blocking pregnancy in prairie voles. She has suggested that urinary compounds signaling "novelty", rather than the conventional idea of "novelty" and "maleness" signals, are sufficient in prairie voles.

One strong argument against adaptive explanations of the Bruce effect in house mice is that the conditions necessary to produce the effect are unlikely to occur in the field (see Bronson & Coquelin, 1980). In general it appears that a pregnancy block can occur under a wider range of conditions in some vole species than in house mice. Thus,

the conditions necessary might be more likely to occur with regularity in the field.

Also during pregnancy, a stud-male mediated maintenance of pregnancy does occur in at least one vole species (montane voles) and may occur in other species (see Chapter 4). This effect has not been reported in house mice. The mechanism may involve an olfactory influence on the maintenance of the corpora lutea (Brown, 1985; see Chapter 4).

Primer actions at the nest site. Mus and Microtus also appear to differ in the action of priming pheromones at the nest site. In house mice, urine from females during the last two-thirds of pregnancy, and the last two-thirds of lactation, accelerates puberty in a manner similar to that of male urine (see Drickamer, 1986). The likely recipients of this acceleration signal are the progeny of the female depositing the urine (Drickamer, 1986). In at least three species of Microtus however, there is evidence that chemosignals released at the nest site can suppress reproductive activation. Lepri (1986) found that reproductive activation of pine vole females was suppressed in the presence of bedding soiled by her family. Rissman et al., (1984) found that family chemical cues delay puberty in male California voles. Getz et al., (1983) found that urine from pregnant prairie voles suppressed reproductive activation in virgin females that had been previously stimulated by males.

Density-dependent primer actions. In other areas there has not been enough investigation among Microtus species to make meaningful comparisons with house mice. One of the most studied mouse primers is the puberty delay signal released by group-housed females. The puberty inhibiting pheromone is present in the bladder urine of all females, but released only by grouped females, thus indicating a urethral gating mechanism. It is density dependent, in that the effectiveness of equal amounts of urine from females living in groups is directly related to group size. It is potent at extremely low concentrations, and can override the accelerating action of males on young females. The puberty delay signal is released under natural conditions at high population densities. For reviews, see Bronson (1979a), Drickamer (1986), and Vandenbergh and Coppola (1986). Vandenbergh and Coppola (1986) stress that puberty inhibition may be critically important under natural conditions.

There have been no really comparable studies of group housing and the production of a delay signal in voles. Batzli et al. (1977) found that littermate prairie and California voles, but not meadow voles, suppressed growth and reproduction in one another. The mechanism of suppression was not identified however, and the methods of the study are rather difficult to understand. As noted above, Getz et al. (1983) found that urine from individually housed pregnant (cf., house mice) and virgin females

suppressed uterine growth in prairie vole females that had been previously activated. It is not known if grouping would alter this effect. No other Microtus species have been studied.

Other primer actions. There are at least two other primer effects identified in house mice that have not received comparable investigation in Microtus. First, urine from house mouse females in estrus accelerates puberty in juvenile females in a manner similar to male urine (see Drickamer, 1986). Second, urine from house mouse females elicits in males an immediate LH increase followed by an elevation in testosterone levels (see Bronson, 1979a; Bronson & Coquelin, 1980). This response is independent of the gonadal condition of females and the sexual experience of males. Because it is independent of the gonadal state of releasing females, Bronson (1979a,b) has proposed this primer serves a key function in allowing mutual stimulation between the sexes. It is not known if either of these effects are present in voles.

Summary. In summary, there are both similarities and differences in the actions of primers in house mice and voles. In both, male produced chemosignals seem to stimulate the reproductive system of females. The results of this stimulation appear to be very similar for juvenile house mice and vole females; earlier puberty. In adult females, at least some Microtus species are much more dependent on contact with male odors for the attainment of

estrus than house mice. Differences in the manifestation of the Bruce effect in Mus and Microtus may indicate underlying differences in mechanism. The infrequently studied pregnancy maintenance phenomenon, present in montane and perhaps other vole species, does not seem to be present in house mice. Chemosignals at the nest site may promote reproductive activation in house mice, whereas chemosignals at the nest site in voles appear to be inhibitory. Finally, for some primer effects, there has not been sufficient study to compare Mus and Microtus. This is probably most critical regarding the puberty delaying effect of grouped females. This phenomenon may be quite important in nature for house mice (Coppola, 1986; Vandenbergh & Coppola, 1986) during population fluctuations. It would certainly seem worthy of study, as students of Microtus have long been interested in explaining the massive density fluctuations in vole populations (see Taitt & Krebs, 1985).

Role of Priming Pheromones in the Reproductive Strategies of Voles

The brain-pituitary-gonadal axis forms the endocrinological core for all mammalian reproduction. There is much interspecies variation in the function of this axis as a result of past selection for reproductive success under different environmental conditions. Adaptations of this axis include responsiveness to environmental cues that signal appropriate or inappropriate conditions for reproduction (Bronson, 1979a). These cues can have direct

(i.e., result in specific neuroendocrine changes) or indirect influences on the axis and hence reproduction. Examples of cues with direct effects include photoperiod and priming pheromones; cues with indirect effects include caloric intake and agonistic stimuli (see Bronson, 1979a). The timing of reproduction in short-lived small mammals with short generation times and uncertain environments is critical. Priming pheromones provide a more favorable signal to noise ratio than other potential indicators (e.g., photoperiod, ambient temperature) for short-lived mammals (see Bronson, 1979a; Negus & Berger, 1987).

The role of priming pheromones in the reproductive strategies of house mice has been the subject of much interest (e.g., Bronson, 1979a,b; Drickamer, 1986; Vandenbergh, 1983; Vandenbergh & Coppola, 1986). Bronson (1979a,b) has argued that the primary function is coordination of ovulation. Coordination of ovulation in relation to dispersal of young animals is likely to have been a fundamental factor in the world-wide colonization success of house mice. He proposes that reproduction is suppressed at the nest site, but upon successful dispersal to a suitable locale reproduction can proceed rapidly. Bronson (1979a) also proposes that pheromonally cued coordination of ovulation is likely to be useful during the opportunistic shifting of home ranges that is common in low density feral populations. Vandenbergh and Coppola (1986), in contrast to Bronson (1979a), argue that pheromonal cueing

is also likely to be of great value in stable, high density populations. In the context of life history strategies, they propose that reproduction is pheromonally modulated in a manner that promotes lifetime reproductive success. Females may accelerate or delay puberty and reproduction dependent on the current chances for reproductive success.

The proposed roles for pheromonal cueing of reproduction in voles are similar to Bronson's (1979a) proposals for house mice; reproductive suppression of young animals at the nest site and promotion of rapid ovulation upon dispersal. Hypothesized benefits of reproductive suppression at the nest site include incest avoidance (Batzli et al., 1977; Carter & Getz, 1985) and avoidance of competition for resources with the primary reproductive pair (Getz & Carter, 1980; Getz et al., 1987). Upon dispersal to a suitable site, male induced estrus and ovulation are thought to promote rapid reproduction only under proper conditions when a male is present (Getz & Carter, 1980; Richmond & Stehn, 1976).

Compared with other common rodents (e.g., rats, mice, hamsters), female voles seem much more responsive and dependent on male chemosignals in all aspects of their reproductive lives. Vole females are highly responsive to male chemosignals at puberty (puberty acceleration), estrus (stimulation of vaginal and behavioral estrus), and during pregnancy (pregnancy maintenance and the Bruce effect). Contact with a male at each major reproductive juncture

influences reproductive success. The presence of male chemosignals may therefore be an indicator of environmental suitability for reproduction. For example, if no healthy, vigorous males are present, environmental conditions are likely to be poor. In this case the female remains reproductively quiescent, conserving energy for future reproduction. The disappearance of the stud, or his replacement by a strange male, may indicate unstable and inappropriate conditions for reproduction. Pregnancies are unlikely to be maintained under these conditions. The continued presence of a healthy stud male indicates good conditions for reproduction. A continuously present stud can induce rapid puberty, estrus, and maintain the pregnancy in a young virgin female.

Density-dependent influences on primer action should receive further study in order to understand the role of priming pheromones in reproductive strategies. Voles are known to show striking cycles in population density (Getz, 1978; Heske et al., 1988; Taitt & Krebs, 1985). Pheromonal cueing may play a part in these cycles (see Heske et al., 1988). At high densities the pheromonal suppression of reproduction at the nest site appears to break down, as young females become reproductively activated in high numbers (Getz & Hofmann, 1986; Getz et al., 1987; Hofmann & Getz, 1988). Whether this is a cause or effect of population cycles is not known (Getz et al., 1987). Other primer effects such as estrus induction, pregnancy

maintenance, and pregnancy interruption, may be altered as a result of shifts in population density. There is not an adequate literature (cf. house mice) on density dependent influences on primer action in Microtus.

Species Differences in Primer Action

Perhaps the most interesting area of future research on priming pheromones in Microtus will focus on species differences in primer actions. Species differences may offer important clues regarding the adaptive function of priming pheromones in natural populations. A number of species differences in primer action have been identified, and it is likely that many more important differences remain unrecognized. Species should ideally be compared under identical conditions to identify differences (see Chapter 3); such a data base is generally not available. Some of the differences in primer responsiveness among Microtus that have been identified, and the possible explanations for such, will be discussed below. Suggestions for future comparative study are also made.

Reproductive suppression among littermates differs in meadow, prairie, and California voles (Batzli et al., 1977). Some authors have related these differences to differences in habitat (Batzli et al., 1977; Lepri, 1986; Vandenbergh, 1986). They note that reproductive suppression is most evident in species that inhabit stable and continuous habitats were opportunities for dispersal are limited. Reproductive suppression would allow a longer period of time

for dispersal. Further comparative study on reproductive suppression is needed to evaluate this hypothesis. Informal observation indicates that montane voles are more likely to inbreed than prairie voles when housed with littermates in this laboratory (Taylor, personal observation). The habitat of montane voles is probably patchy rather than stable and continuous (Getz, 1985). There are however many difficulties determining the "patchiness" of a habitat and comparing species on this measure (see Getz, 1985).

The potential relationship between responsiveness to primers and social organization and mating systems has been emphasized in the present work. At a very basic level, more social species should be more responsive to primer actions, and the value of such capacities more relevant, as the presence of conspecifics is likely to be a more important part of the environment than asocial species (Levin & Johnston, 1986). Beyond this basic level, there may be further "tuning" of the pheromonal cueing system in relation to the specifics of the social structure and mating system of the species in question.

In Chapter 3 differences among montane, meadow, and prairie voles were found in primer responsiveness; the duration of male exposure needed for receptivity in females. Such differences may be widespread among Microtus (see Chapter 3 for review). As discussed in Chapter 3, these differences may be related to social organization and mating systems. Estrus induction requiring long durations of male

exposure may be associated with social species that display monogamous mating systems. The long period of exposure necessary may limit a male regarding the number of females he can find and induce, thus predisposing monogamy. Also, the long period of estrus induction may afford the female greater opportunity for mate evaluation. Voles that display monogamous mating systems generally display mate choice more readily than polygamous species (e.g., Pierce, 1989; Pierce et al., 1989; Shapiro & Dewsbury, 1986; Shapiro et al., 1986). Males should be less constrained in their ability to induce and mate with multiple females in species that do not require a long period of male exposure. This type of physiology should be found in asocial polygamous species.

Stud-male mediated maintenance of pregnancy (see Chapter 4) may also vary with social organization and mating system, as it is a primer dependent on post-copulatory interactions between the stud and female. Although both monogamous and polygamous species may show post-copulatory contact with the stud, it differs in degree (see Chapter 4). Further work might show, for example, that social species that form monogamous pairbonds require more post-copulatory contact for pregnancy maintenance than asocial polygamous species.

Another primer effect worthy of further comparative investigation is male stimulated puberty acceleration. Although demonstrated in a number of vole species, differing experimental methodologies and measures of acceleration make

comparisons across studies difficult (Sawrey, 1989; Sawrey & Dewsbury, submitted). Despite these difficulties, some interesting differences appear to exist (Sawrey & Dewsbury, submitted). Meadow vole females show reproductive activation across double layer wire-mesh barriers (Baddaloo & Clulow, 1981). Prairie voles do not show reproductive activation unless allowed direct contact with males or chemical stimuli from males (Carter et al., 1980; Carter et al., 1987). Sawrey and Dewsbury (submitted) have noted these differences in the stimuli required for reproductive activation are interesting in light of the respective mating strategies of each species. Requiring contact for activation may facilitate mate choice. Prairie voles are monogamous and females appear to be "choosey" regarding mates (e.g., Pierce et al., 1989; Shapiro & Dewsbury, 1986; Shapiro et al., 1986), whereas meadow voles may be less so (Pierce et al., 1989). It may also be the case that some species (e.g., more social species) are more labile with regard to acceleration, whereas other species may approach the physiological "limit" (see Levin & Johnston, 1986) to early puberty. In other words, the lability of puberty onset may vary with the degree of sociality. Solid evidence of species differences in lability could only be obtained by comparing species under identical conditions; this has not been attempted.

Social organization and mating systems differences may be associated with species differences in manifestation of

the Bruce effect. As noted above, prairie and field voles differ in the stimuli sufficient for pregnancy interruption. Kenney et al., (1977) found lower rates of post-implantation disruption in meadow than prairie voles. Heske (1987) noted California voles require a longer period of strange-male exposure for pregnancy interruption than prairie voles. Thus it may be that monogamous prairie voles are more sensitive to pregnancy interrupting stimuli than meadow, California, and field voles, which are polygamous (see Table 3-14). Again however, differing methodologies make comparisons across studies difficult.

Thus it appears that species differences in primer responsiveness is fertile ground for further comparative study in Microtus. There is an opportunity to compare many species which differ in important aspects of behavior and social organization. All these species are likely to be highly responsive to pheromonal cueing. Subtle differences in primer responsiveness exist however, and are likely to contribute to species differences in behavior. With further study we can learn more about the causes of species differences in behavior and the adaptive function of pheromonal cueing systems in nature.

Final Remarks

In the last thirty years our knowledge of the influence of chemosignals on mammalian reproduction has exploded. The availability of house mice as an ideal model system for studying primer actions has undoubtedly facilitated this

process. The convergence of work from many productive investigators has been illuminating. Primer effects, the stimuli that cause them, and the underlying neuroendocrine mechanisms are all fairly well understood in house mice; more so than any other mammal. It has however, resulted in a rather biased view of mammalian reproduction and the role of chemosignals (see Bronson, 1979b, 1987, 1989).

Further advances in our understanding of the role of chemosignals in mammalian reproduction are likely to come with a broader approach. The picture of primer actions available for house mice is clearly not the only picture. Many other species are quite responsive to primers and are likely to use them in different ways. The primer phenomena studied in this dissertation are good examples. There are many interesting questions of mechanism and function that remain to be investigated. In the future work that adds to the comparative data base will be especially valuable in contributing to a more complete understanding of the role of chemosignals in mammalian reproduction.

APPENDIX A
VALUES OF U (MANN-WHITNEY TEST) FOR PAIRWISE COMPARISONS OF VAGINAL SMEAR CELL TYPES

	Day		
Part A (nulliparous)	1	2	3
Cornified Cells			
Mont vs. Pr(S1)	70.0	64.5	62.5
Mont vs. Mead	158.0	219.0	199.0
Mont vs. Pr(S2)	62.5	46.5	37.5
Pr(S1) vs. Mead	43.0	44.0	56.5
Pr(S1) vs. Pr(S2)	52.5	89.0	53.5
Mead vs. Pr(S2)	39.5	31.5	30.0
Nucleated Cells			
Mont vs. Pr(S1)	90.0		
Mont vs. Mead	90.0		
Mont vs. Pr(S2)	27.0		
Pr(S1) vs. Mead	152.0		
Pr(S1) vs. Pr(S2)	63.0		
Mead vs. Pr(S2)	67.5		
Leukocytes			
Mont vs. Pr(S1)	73.5	65.0	63.5
Mont vs. Mead	166.0	225.0	194.5
Mont vs. Pr(S2)	58.0	46.0	37.0
Pr(S1) vs. Mead	55.0	32.0	54.0
Pr(S1) vs. Pr(S2)	56.5	92.5	49.0
Mead vs. Pr(S2)	39.5	24.0	26.0
Part B (parous)			
Cornified Cells			
Mont vs. Pr(S1)	20.0	40.0	29.0
Mont vs. Mead	44.0	48.5	54.0
Mont vs. Pr(S2)	17.0	22.5	20.0
Pr(S1) vs. Mead	19.0	43.5	29.5
Pr(S1) vs. Pr(S2)	39.0	46.5	47.5
Mead vs. Pr(S2)	13.0	21.0	23.0

Nucleated Cells			
Mont vs. Pr(S1)			
Mont vs. Mead			
Mont vs. Pr(S2)			
Pr(S1) vs. Mead			
Pr(S1) vs. Pr(S2)			
Mead vs. Pr(S2)			
Leukocytes			
Mont vs. Pr(S1)	24.0	36.5	27.0
Mont vs. Mead	44.0	51.5	53.5
Mont vs. Pr(S2)	15.5	22.0	20.0
Pr(S1) vs. Mead	22.0	43.5	29.5
Pr(S1) vs. Pr(S2)	37.5	46.5	46.0
Mead vs. Pr(S2)	13.0	20.0	23.0

Mont = montane voles; Mead = meadow voles; Pr(S1) = squad I prairie voles; Pr(S2) = squad II prairie voles

APPENDIX B
VAGINAL SMEARS OF NULLIPAROUS AND PAROUS FEMALES FOR DAYS 1, 2, AND 3

		Categories						
	N	0	2	4	6	8	SP	NUC
Nulliparus								
Day 1								
Mont	22	6	6	3	3	2	2	1
Mead	21	3	6	3	2	4	3	0
Pr(S1)	23	12	3	2	0	0	6	1
Pr(S2)	12	5	2	1	0	1	3	0
Day 2								
Mont	22	5	2	3	4	8	0	1
Mead	21	5	4	3	4	5	0	0
Pr(S1)	23	14	3	0	0	0	6	0
Pr(S2)	12	8	2	1	0	0	1	0
Day 3								
Mont	22	4	3	3	5	6	1	0
Mead	21	5	7	0	6	3	0	0
Pr(S1)	23	11	2	2	0	0	8	1
Pr(S2)	12	8	1	0	0	0	3	0
Parous								
Day 1								
Mont	10	4	3	2	0	1	0	0
Mead	13	3	3	1	4	2	0	0
Pr(S1)	12	7	2	1	0	0	2	1
Pr(S2)	9	7	2	0	0	0	0	0
Day 2								
Mont	10	4	2	3	0	1	0	0
Mead	13	4	0	4	3	2	0	0
Pr(S1)	12	9	1	0	0	2	0	0
Pr(S2)	9	5	3	0	0	0	1	0
Day 3								
Mont	10	2	3	3	1	1	0	0
Mead	13	4	1	2	2	4	0	0

		Categories						
	N	0	2	4	6	8	SP	NUC
Parous								
Day 3								
Pr(S1)	12	9	0	3	0	0	0	0
Pr(S2)	9	5	2	1	0	0	1	0

0 = 0-19% cornified; 2 = 20-39% cornified; 4 = 40-59% cornified; 6 = 60-79% cornified; 8 = 80-100% cornified; SP = "sparse" smear (insufficient cells); NUC = greater than 15% nucleated cells; Mont = montane voles; Mead = meadow voles; Pr(S1) = squad I prairie voles; Pr(S2) = squad II prairie voles

APPENDIX C

RANKS OF VAGINAL SMEARS TAKEN PRIOR TO COPULATION BASED ON PERCENTAGE OF CORNIFIED CELLS

	Day				
Nulliparous	3	4	5	6	7
Mont					
	1	2			
	1	2			
	1	2			
	2	1	3		
	1	2			
	1	4	2	3	
	1	2			
	1	2			
	2	1			
	2	1			
	2	1	3		
Mead					
	1	2			
	1	2			
Pr(S1)					
	2	1	3		
	1	3	2		
	2	1	3		
	1	2	4	3	
	2	1	3	4	
	2	1	3		
	1	2	3		
	3	2	1		
Pr(S2)					
	2	3	1		
	2	1			
	2	1	3	4	
Parous					
Mont					
	1	2			
	3	4	2	1	5
	2.5	2.5	1		

	Day				
Parous	3	4	5	6	7
Mead					
	1	2			
	1	2			
	1	2			
Pr(S1)					
	2	1	3		
	2	1	3		
	2	5	1	4	3
	1	2	3		
	3	1	2		
	1	2	3		
	2	1	3		
	1	2			
	2	1	3		
Pr(S2)					
	1	2	3		
	1	2	3		
	1	2	3		
	1	2	3		

Mont = montane voles; Mead = meadow voles; Pr(S1) = squad I prairie voles; Pr(S2) = squad II prairie voles

APPENDIX D
SMEARS TAKEN FROM NULLIPAROUS AND PAROUS FEMALES
ON THE DAY OF COPULATION

		Categories						
	N	0	2	4	6	8	SP	NUC
Nulliparous								
Mont	14	1	0	3	1	9	0	0
Mead	2	0	0	1	1	0	0	0
Pr(S1)	8	0	2	2	2	2	0	0
Pr(S2)	3	2	0	1	0	0	0	0
Parous								
Mont	8	1	1	3	0	3	0	0
Mead	9	1	1	0	3	4	0	0
Pr(S1)	8	2	1	1	1	3	0	1
Pr(S2)	4	0	0	3	0	1	0	0

0 = 0-19% cornified; 2 = 20-39% cornified; 4 = 40-59% cornified; 6 = 60-79% cornified; 8 = 80-100% cornified; SP = "sparse" smear (insufficient cells); NUC = greater than 15% nucleated cells; Mont = montane voles; Mead = meadow voles; Pr(S1) = squad I prairie voles; Pr(S2) = squad II prairie voles

APPENDIX E
EFFECTS OF PARITY ON PERCENTAGE OF CORNIFIED CELLS IN VAGINAL SMEARS

Species	Day	N	Mean Nulliparous	Mean Parous	T	p
Mead	1	10	52.2	53.3	26.0	n.s.
	2	13	50.3	48.5	43.0	n.s.
	3	13	43.1	56.3	31.0	n.s.
Mont	1	10	36.1	32.7	22.5	n.s.
	2	10	65.3	33.0	7.0	$p < .05$
	3	10	52.9	40.5	17.0	n.s.
Pr(S1)	1	7	12.3	14.6	11.0	n.s.
	2	8	9.3	29.9	9.5	n.s.
	3	7	16.3	12.4	9.0	n.s.
Pr(S2)	1	6	31.2	8.8	4.0	n.s.
	2	7	15.7	14.6	12.0	n.s.
	3	7	14.0	16.6	13.5	n.s.

Mont = montane voles; Mead = meadow voles; Pr(S1) = squad I prairie voles; Pr(S2) = squad II prairie voles

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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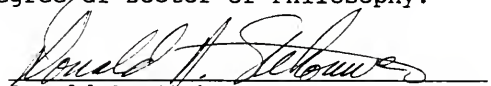

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This dissertation was submitted to the Graduate Faculty of the Department of Psychology in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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