VERHALTENSÖKOLOGISCHE UNTERSUCHUNG AN WALDMÄUSEN (*APODEMUS SYLVATICUS*)

BEHAVIORAL ECOLOGY STUDIES OF WOOD MICE (*APODEMUS SYLVATICUS*)

Dissertation
zur Erlangung des akademischen Grades
des Doktors der Naturwissenschaften (Dr. rer. nat.)
an der
Mathematisch-naturwissenschaftlichen Sektion
Fachbereich Biologie, Universität Konstanz

Vorgelegt von
KERSTIN MUSOLF
Tag der mündlichen Prüfung: 15.10.2002
1. **Gutachter: Prof. Dr. Gabriele Gerlach**
2. **Gutachter: Prof. Dr. Peter Berthold**

TABLE OF CONTENTS

ZUSAMMENFASSUNG

Zur Untersuchung der Evolution von Entscheidungen bei der Wahl von Partnern und Helfern bei Waldmäusen (*Apodemus sylvaticus*), wurde in dieser Studie eine Kombination von Experimenten genutzt, die zum Teil im Labor und zum Teil in einem großen Freigehege durchgeführt wurden.

In Kapitel 1 wurde die Fähigkeit von Waldmausweibchen getestet, Verwandte zu erkennen und der der Erkennung zugrunde liegende Mechanismus wurde untersucht. Der Östrus, der paarungsbereite Zustand im weiblichen Fortpflanzungszyklus, war der Auslöser des weiblichen Paarungsverhaltens. Weibchen in anderen Stadien des Zyklus zeigten ein davon verschiedenes Verhalten. Daraus ließ sich der Schluss ziehen, dass ein spezifisches Paarungsverhalten der Weibchen existiert. Weibchen zeigten eine Präferenz für Gerüche von unbekannten, unverwandten Männchen im Vergleich zu Gerüchen von bekannten, und sogar unbekannten, Brüdern. Der fehlende Bedarf einer Bekanntschaft für die Identifizierung von Verwandten legt nahe, dass das „Phänotyp-Matching“ der Mechanismus der Diskriminierung ist. Ein weiterer Hinweis, ob der Mechanismus des „Phänotyp-Matching“ auf einem Vergleich des eigenen Phänotyps oder des der Familie mit dem Fremd-Phänotyp beruht, konnte mit diesem experimentellen Ansatz nicht erklärt werden. Zusammengefasst lässt sich feststellen, dass Weibchen verwandte Männchen in der Partnerwahl vermeiden.

In Kapitel 2 wurden mögliche Kriterien der Partnerwahl bei Waldmausweibchen untersucht. Interessanterweise zeigten die Weibchen weder für schwere noch für dominante Männchen eine Präferenz. Es handelt sich dabei um Merkmale, die allgemein von weiblichen Nagetieren bevorzugt werden. Außerdem, und im Gegensatz zu früheren Studien an Kleinsäugern, wurde bei Waldmausmännchen keine Beziehung zwischen Körpergewicht, sozialer Dominanz und Urinierungs-Muster gefunden. Ferner kopierten die Weibchen auch nicht das Paarungsverhalten anderer Weibchen; allerdings vermieden sie den kombinierten Geruch eines Paares. Die Vermutung liegt nahe, dass das beinahe willkürliche Paarungsverhalten der Weibchen bedingt wird durch das promiskuitive Paarungssystem dieser Art. Weibchen verpaaren sich gewöhnlich mit mehreren Partnern in jeder Östrus Periode. Der höhere Fortpflanzungserfolg von schwereren Männchen, der in früheren Studien und auch dieser Studie beobachtet wurde, könnte dann nicht als Resultat weiblicher

Wahl erklärt werden, sondern aufgrund unterschiedlicher Sperma-Menge oder Qualität dieser Männchen oder Spermienkonkurrenz. Das Fehlen einer Präferenz der Weibchen für jedes männliche Merkmal in dieser Studie könnte erklären, warum Männchen keine deutlichen Qualitätsanzeichen wie zum Beispiel Urinierungsmuster ausprägen. Weibchen vermeiden andere Weibchen, ein Hinweis auf starke Konkurrenz um Nestplätze, welche der limitierende Faktor für den Fortpflanzungserfolg sind.

In Kapitel 3 wurde die Rolle des „Major Histocompatibility Complex“ (MHC) bei der weiblichen Partnerwahl sowohl im Labor als auch im Freigehege studiert. Unter der Annahme, dass bezüglich des MHCs heterozygote Individuen einen Vorteil bei der Abwehr von Pathogenen haben und Verwandte häufig dieselben MHC Allele teilen (Inzuchtvermeidung), wurde ein MHC-disassortatives Wahlverhalten vorhergesagt. Das bedeutet, dass Weibchen ein Männchen mit einem anderen MHC Typ als den ihren wählen. Proteine des MHC, die unverwechselbare Körpergerüche bedingen, ermöglichen die individuelle Unterscheidung. Die Variation von zwei MHC class II Genen wurde untersucht. Die analysierten Gen-Segmente hatten eine niedrige Variabilität (3–6 Allele) im Vergleich zu anderen Säugetier Arten. Im Gegensatz zu der obigen Annahme bevorzugten die Weibchen nicht den männlichen Genotyp, der von ihrem verschieden war, aber auch nicht den, der ihrem glich. Weiterhin konnte kein Hinweis für eine Präferenz für Männchen mit mehr Allelen pro Locus gefunden werden. Diese Ergebnisse passen zu dem bereits gefundenen willkürlichen Partnerwahlverhalten von Waldmausweibchen aus dem vorherigen Kapitel.

Kapitel 4 behandelt die Gründe für die mehrfachen Verpaarungen der Weibchen. In einem polyandrischen, experimentellen Aufbau konnte gezeigt werden, dass einzeln gezeugte Würfe häufiger unter Infantizid litten als Würfe, die von mehreren Männchen gezeugt worden waren. Die Weibchen reagierten aktiv auf den männlichen Infantizid durch die Auswahl von mehreren Partnern, wenn der frühere Wurf getötet worden war. Folglich scheint Promiskuität eine Gegenstrategie der Weibchen gegen Infantizid der Männchen zu sein, indem dadurch die Vaterschaft verschleiert wird.

Zur Bestimmung der Vaterschaften der Waldmaus Jungtiere wurden Mikrosatelliten Analysen benutzt. In Kapitel 5 werden die Entwicklung und Charakterisierung von 6 neuen Mikrosatelliten Primer Paaren für Waldmäuse für weiterführende Analysen vorgestellt.

Schließlich wurde in Kapitel 6 die Verhaltensökologie von Waldmäusen und besonders das weibliche Sozialverhalten unter semi-natürlichen Bedingungen in einem großen Freigehege untersucht. Die gefundene hohe Anzahl an multiplen Vaterschaften bestätigt das promiskuitive Paarungssystem dieser Art. Das Körpergewicht der Männchen hatte den größten Einfluss auf den männlichen Fortpflanzungserfolg. Hier konnte ebenfalls festgestellt werden, dass schwerere Männchen mehr Nachkommen zeugten. Der Fortpflanzungserfolg der Weibchen korrelierte positiv mit ihrem Aufenthalt im Gehege. Vermutlich war dieses mit der Nestplatz-Verfügbarkeit verknüpft. Weibchen, die mit einer Limitierung der Nestplätze umgehen mussten, zeigten eine alternative Strategie - das Erben des Nestplatzes / Territoriums. Obwohl der Aufenthalt im mütterlichen Gebiet mit einer reproduktiven Unterdrückung verbunden war, lieferte die Möglichkeit, ein Territorium hoher Qualität zu erben, ausreichenden Nutzen im Hinblick auf gesteigerten zukünftigen Fortpflanzungserfolg.

SUMMARY

In order to investigate the evolution of mate choice decisions and helper choice in wood mice (*Apodemus sylvaticus*), this study uses a combination of experiments performed in the laboratory and in a large outdoor enclosure.

In Chapter 1, the capability of female wood mice to recognize kin and mechanism involved in kin recognition were investigated. The estrus, the female's status of reproduction, was very important in triggering female's mating behavior. Therefore, it is considered that a distinct mate choice behavior exists. Females showed a preference for unfamiliar unrelated male odors compared to odors of familiar and even unfamiliar brothers. The lack of familiarity needed to identify kin suggests that phenotype matching is being used. Further delineating whether phenotype matching is based on self-matching or familial imprinting could not be addressed with this experimental approach. To sum up, females avoided related males in mate choice decisions.

In Chapter 2, possible mate choice criteria of female wood mice were investigated. Interestingly females showed no preference neither for heavier nor for dominant males, traits which are generally preferred in female rodents. Also, in contrast to previous studies on other small mammals, no relationship between body weight, social dominance, and urination patterns in male wood mice was shown. Furthermore, females did not copy pairing behavior of another female, but avoided the combined odor of a pair. I assume that this nearly indiscriminate mating behavior is caused by the promiscuous mating system in this species. Females mate in general with multiple partners for each estrus cycle. The higher reproductive success of heavier males found in previous and also this study could then be explained not as a result of female choice but in terms of different sperm quantity or quality or with sperm competition. The lack of preference for any male quality trait in this study might explain why males do not exhibit distinct quality signs such as urinary pattern. Females avoided other females, indicating high competition for nest site availability is the limiting factor for reproductive success.

In Chapter 3 the role of the major histocompatibility complex (MHC) in female mate choice selection was investigated in the lab and a large enclosure. MHC-disassortative mating has been predicted, assuming the operation of pathogen-driven MHC heterozygote advantage and/or inbreeding avoidance due to kin sharing the same alleles. Individual discrimination maybe facilitated by distinctive odors produced by MHC proteins. In this chapter, variation at two MHC class II genes was examined. The amplified gene segments had a low variability (3–6 alleles) compared with other mammalian species. In contrast to the expectation, females displayed no preference for male genotypes dissimilar or similar to their own. There is also no evidence for a preference for males with a higher number of alleles per locus. These results are consistent with the indiscriminative female mate choice as shown in the former chapter.

Chapter 4 addressed the reasons for female multiple-mating behavior. In a polyandrous experimental set-up, it has been found that single-sired litters suffered male infanticide more often than litters that were sired by more than one male. Females reacted actively to male infanticide by choosing more partners if the previous litter had been killed. Promiscuity therefore appears to be a counter strategy of females against infanticidal males through the confusion of paternity.

To determine paternity of wood mouse offspring, microsatellite analyses have been used. The development and characterization of six new primer sets for wood mice for further analyses has been introduced in Chapter 5.

Finally, in Chapter 6, wood mice behavior and especially female social behavior has been studied under semi-natural conditions in a large enclosure. The high frequency of multiple paternities found confirms the promiscuous mating system in this species. Male body weight had the strongest impact on male’s reproductive success, also demonstrating that heavier males sire more offspring as mentioned above. Female reproductive success was positively correlated with their stay in the enclosure and this was considered to be linked with nest site availability. Females who had to deal with limited nest sites showed an alternative strategy of nest/territory inheritance. Although staying in the mother’s territory was associated with reproductive suppression, the possibility of inheriting a high quality territory provided sufficient benefits in terms of increased future reproductive success.

INTRODUCTION

Behavioral ecology investigates why (ultimate causes) and how (proximate causes) the various behavioral characteristics of animals have evolved, as well as the evolution of the mechanism of behavior. Insights gained from investigations of animal behaviors have contributed to our understanding of natural and sexual selection in wild populations.

Natural and sexual selection are the driving forces in evolution and provide insight into why organisms are what they are. Natural selection is defined as the differential survival and/or reproduction of classes of entities that differ in one or more characteristics whereas sexual selection is defined as differential reproduction owing to variation in the ability to obtain mates. Darwin was convinced that natural selection alone could not bring about such conspicuous traits such as the bright colors, horns and displays of males of many species, but instead posed an alternative selective force, sexual selection (Darwin, 1871). Sexual selection may act at both intrasexual and intersexual levels. The contest between males for access to females is the intrasexual force; female choice or preferences of some male phenotypes over others drives the intersexual selection.

Sexual selection exists because females produce few large gametes and males produce many small gametes. The costs to produce these gametes, eggs and sperm, are different. In mammals females produce several hundred cells throughout a lifespan whereas a male can produce billions of sperms in his life. This creates an automatic conflict between the reproductive strategies of the sexes: a male can mate with many females, and often suffers little reduction in fitness if he should mate with an inappropriate female, whereas all eggs can be fertilized by a single male (usually), and the female’s fitness can be significantly lowered by inappropriate matings (Trivers, 1972). Commonly then, females are a limiting resource for males, which compete for mates, but males are not a limiting resource for females. With this assumption the power of female mate choice leads to the expression of diverse traits such as the elaborate trains of peacocks (Petrie, 1994), the hyper-developed tail fins forming the sword of swordtails (Basolo, 1990), the intricately constructed bowers of bower birds (Borgia, 1985) and the huge eye spans in stalk-eyed flies (Wilkinson & Reillo, 1994).

How distinct the effects or the power of female mate choice are depends on the mating system. In harems for example, in which several females are dominated by a single male, an individual female does not generally have a choice with whom she would like to mate apart from the holder of the harem. But in a monogamous system with free female choice the female's selection has more influence.

Mating systems

Cases where mates usually maintain exclusive or semi-exclusive mating bonds with a single partner throughout most of their lifespan are called long-term monogamy. If the mating bond remains stable for a single breeding attempt but a different partner will be chosen for the next season the system is termed serial monogamy. Polygyny or harems exist when males mate with a group of females, whereas the opposite pattern, a single female with a group of males is called polyandry. When males mate with any receptive female and there is no continuing bond between individual males and females after mating has occurred, the system is a promiscuous one. Females can also mate promiscuously with several males over a single estrus period, resulting in multiple paternities within one litter. Male mammals show a diverse array of mating bonds, including obligate monogamy, unimale and group polygyny and promiscuity. These are associated with a wide variety of different forms of mate guarding, including the defense of feeding and mating territories, the defense of female groups and the defense of individual receptive females (Emlen & Oring, 1977; Clutton-Brock, 1989). Mating bonds in female mammals include long-term monogamy, serial monogamy, polyandry and promiscuity. Both male and female mating behavior varies widely within species. In mammals, the males of more than 90% of all species are habitually polygynous (Kleiman, 1977; Rutberg, 1983). While philandering and extra fertilizations yield clear benefits to polygynous males, it has long been assumed that females do not benefit from multiple mating and, as a result, monogamy has been considered the default state in females. The increased use of molecular techniques has revealed moderate to high levels of multiple paternity in a wide range of animal groups (taxonomic reviews in Birkhead & Møller, 1998) and a substantial body of recent work has been focused on the benefits of female multiple mating (Hrdy, 2000;

Jennions & Petrie, 2000).

Female mate choice

Why do we study female mating preferences and mate choice? Jennions & Petrie (1997) provided several reasons (evolutionary history, ecological / social / morphological factors, neurobiology and psychology of female choice). The present study focuses on the influence of female mating preferences on the rate and direction of evolution by sexual selection.

There are numerous suggestions as to what females gain by being selective in their choice of mates (Kirkpatrick & Ryan, 1991; Andersson, 1994). Females can gain not only benefits such as essential territorial resources, paternal care or food gifts, but also their immediate reproductive success, fecundity and/or the quality of offspring, all of which can vary among males (Drickamer et al., 2000).

How can females assess the quality of a male? The presentation of material benefits such as food may be a good criterion as these gifts can be directly translated into fitness benefits in females. Some male hanging flies, katydids, and crickets gather, process, and transfer food, termed a nuptial gift, directly to a discriminating partner. It has been shown that the duration of mating and consequently the amount of sperm a male is able to transfer is dependent on the size of the nuptial gift in the hanging fly (*Bittacus apicalis*) (Thornhill, 1976). Females may also be able to improve their fecundity by feeding on nuptial gifts. Female katydids (*Requema verticalis*) produce more eggs when fed more or larger spermatophores, containers for sperm that are eaten by the female after mating (Gwynne, 1984). In both of these examples, males are not allowed to transfer sperm until a nuptial gift is provided.

Territories may also provide benefits such as adequate food resources to raise offspring successfully and/or better nest sites. In birds the reproductive success of owners of a higher quality territory is increased (Krebs, 1971; Dhondt & Schillemans, 1983). Recent studies on great reed warblers (*Acrocephalus arundinaceus)* (Bensch & Hasselquist, 1992) and bullfrogs (*Rana catesbiana)* (Howard, 1980) demonstrate that females often choose males with higher quality territories.

Paternal care may also a very important quality criterion, especially in species where a female is not able to raise the offspring alone or provides no maternal care. There are many different aspects of male appearance and behavior that might signal male parental capacity. For example, females of some fishes with paternal males apparently prefer individuals that already have eggs in their nests (Unger & Sargent, 1988), perhaps because the eggs demonstrate parental commitment (but see Jamieson & Colgan, 1989). Female fish might also be able to use other indicators of male parental ability in picking a superior caretaker for their offspring. For example, they might prefer large males, because they could be more aggressive, protective parents than small ones would be (Noonan, 1983).

In species in which males do not provide parental care or material benefits, females might choose mating partners whose genes will confer greater fitness (including mating success) on the female's offspring ('good genes' hypothesis) (Møller & Alatalo, 1999). Females could use any male trait correlated with fitness as a guide to advantageous mating. Differences in male quality could be assessed by chemical signaling, physical strength, ornaments and parasite load (Milinski & Bakker, 1990; Møller, 1990). There is ample empirical evidence for female choice based on male traits (such as the spur length of male pheasants (*Phasianus colchicus*) (von Schantz et al., 1989), the brighter coloration of male sticklebacks (*Gasterosteus aculeatus*) (Milinski & Bakker, 1990) and male house finches *(Carpodacus mexicanus)* (Hill, 1990)) that reflect vigor and recent studies indicate that females can increase the survival of their offspring by mating with a high quality male (Norris, 1993; Petrie, 1994; von Schantz et al., 1994; von Schantz et al., 1996; Hasselquist et al., 1996).

The male's dominance status might be also a choice criterion. In small mammals, higher body weight is indicative of dominance (Wolff, 1985b but see Gosling et al., 2000). Barnard & Fitzsimons (1989) found a significant correlation between male rank and body weight in laboratory mice with heavier males having higher rank. Another good predictor of adult (*Mus musculus*) dominance status is the rate of scent marking: males that marked more than their brothers were more likely to become dominant (Collins et al., 1997). The urine of male mice contains major urinary proteins whose function may be to mediate the controlled release of pheromones in scent marks (Hurst et al., 1998). The quality of an individual's odor can allow potential mates to discriminate against individuals of low social status, poor health status or unsuitable genotype (reviewed by Brown & Macdonald, 1985). Competitive scent marking provides a further mechanism to allow females to discriminate between males of apparent high quality (Rich & Hurst, 1998).

Variation at the major histocompatibility complex (MHC) has also been shown be an important measure of male quality (Penn & Potts, 1999). The highly polymorphic genes of the MHC control immunological self/nonself-recognition by coding for cell-surface proteins that bind peptides from invading pathogens and present them to cells of the immune system. Numerous studies have shown that MHC genotype is associated with the production of distinctive odors used in the individual discrimination by mice, rat and humans (reviewed by (Brown & Eklund, 1994). The mechanism by which MHC type influences urine odor is still unclear and appears to involve microorganisms in same cases (Singer et al., 1997; Singh, 2001). There are three adaptive hypotheses for MHC-dependent mating preferences. First, MHC-disassortative mating preference produce MHC-heterozygous offspring that may have enhanced immunocompetence. Although this hypothesis is not supported by tests against single parasites, MHC heterozygotes may be resistant to multiple parasites (Apanius et al., 1997). Second, MHC-dependent mating preferences enable hosts to provide a “moving target” against rapidly evolving parasites that escape immune recognition (the Red Queen hypothesis). Such parasites are suspected to drive MHC diversity through rare-allele advantage (reviewed in Penn & Potts, 1999). Thus, the two forms of parasite-mediated selection thought to drive MHC diversity, heterozygote and rare-allele advantage, will also favor MHC-dependent mating preferences. Finally, because MHC genes are highly polymorphic, individuals sharing MHC alleles are likely to be related. Therefore MHC-dependent mating preference may function to avoid kin matings and deleterious consequences of inbreeding (Potts & Wakeland, 1993). This hypothesis is consistent with additional evidence that MHC genes play a role in kin recognition. In numerous studies in mice and humans, mating preferences appear to be MHC-disassortative (i.e. the test individual prefers individuals with a MHC genotype different from their own), with no general preference or avoidance of specific MHC haplotypes (Yamazaki et al., 1976; Yamazaki et al., 1978; Yamazaki et al., 1988; Beauchamp et al., 1988; Egid &

Brown, 1989; Potts et al., 1991; Wedekind et al., 1995; Ober et al., 1997). In mice, this preference can be influenced by cross-fostering (Eklund, 1997a; Eklund, 1999) or male dominance (Egid & Brown, 1989). Parental sharing of certain MHC alleles correlates with frequent spontaneous abortions or prolonged intergestational intervals in mice, humans and pigtailed macaques (Warner et al., 1991; Jin et al., 1995; Ober, 1995; Knapp et al., 1996). Exposing pregnant female mice to urine of a foreign male possessing MHC alleles different from those of the father led to an increase in resorption of the fetus compared with a confrontation involving two MHC-identical males (Yamazaki et al., 1986).

Females may also choose their mates by copying mate choice decisions of other females (Gibson & Höglund, 1992; Pruett-Jones, 1992). Copying behavior has been demonstrated in several species of fish, including the guppy (*Poecilia reticulata*) (Dugatkin & Godin, 1992), the Japanese medeka (*Oryzias latipes*) (Grant & Green, 1996), the sailfin molly (*Poecilia latipinna*) (Schlupp & Ryan, 1996) and in several lekking species of birds such as the Japanese quail (*Coturnix coturnix*) (Galef & White, 1998), the sage grouse (*Centrocercus urophasianus*) (Gibson et al., 1991), the black grouse (*Tetrao tetrix*) (Höglund et al., 1995) and also in fallow deer (*Dama dama*) (Clutton-Brock et al., 1989). In all of these species males do not provide parental care. Copying could serve either to increase the accuracy of mate assessment or to reduce its costs.

Avoidance of inbreeding

Finally, females should avoid close kin as mating partners. Mating among relatives is assumed to be under strong selection pressure, as inbreeding decreases fertility and survival of offspring (Ralls et al., 1986; Charlesworth & Charlesworth, 1987). Although few data from natural populations are available, studies of captive and experimental animals consistently confirm the ubiquity and magnitude of inbreeding effects (Ralls & Ballou, 1983). Rodents have been key in the study of inbreeding effects. In studies of laboratory mice and the vlei rat (*Otomys irroratus*), litter sizes are reduced when parents are closely related (Barnard & Fitzsimons, 1989; Pillay, 1998). Inbreeding of oldfield mice (*Peromyscus polionotus*) has been shown to have several negative effects: decrease of fertility and production of a lower number of offspring, who were also reduced in viability and mass at weaning (Lacy et al., 1996). Additionally, Margulis (1998) demonstrated that inbreeding in oldfield mice affects the reproductive success of inbred adults. Further detrimental effects have been found when inbred and outbred animals have to compete directly under semi-natural conditions. Inbred male mice sired only one-fifth as many surviving offspring as outbred males because of their poor competitive ability and survivorship (Meagher et al., 2000). Inbred white-footed mice (*Peromyscus leucopus noveboracensis*) suffered lower survivorship than non-inbred ones (Jiménez et al., 1994) when released back into the field site from which the progenitors had been captured.

When sexually mature relatives occur within the same social group or local area, the potential for matings between them exists. However, long-term field studies indicate that such matings occur less often than one might expect (McCracken & Bradbury, 1977; Schwartz & Armitage, 1980; Hoogland, 1982). This implies the existence of the ability to recognize actual of probable kin and avoid them as mating partners as has been shown in several species (Wilmsen Thornhill, 1993), but the mechanisms individuals use for differentiating kin from non-kin are less clear.

Kin recognition

Kin recognition, the determination of genetically related individuals and showing differential behavior towards the conspecific, is an important ability, especially with regard to social behavior. On the one hand, kin recognition may facilitate preferential treatment of kin, such as cooperation in food finding or in warning against predators, and therefore those individuals behaving altruistically in kin groups can increase their inclusive fitness. On the other hand, kin should be avoided as mates to prevent inbreeding.

Several different recognition mechanisms exist for the identification of kin: (1) spatial location; (2) association or familiarity; (3) phenotype matching; and (4) recognition alleles. The basis of all these mechanisms is learning (Tang-Martinez, 2001), just the cues that are used to discriminate between kin and non-kin are different.

The “spatial location mechanism” depends on a close and consistent correlation between genetic relatedness and spatial distribution. Cues based on location (e.g. nest site) are recognized rather than conspecifics per se. This phenomenon is often found in birds, where avian parents recognize their nest site rather than chicks themselves (Hoogland & Sherman, 1976). Therefore, this mechanism should not be considered as a real kin recognition mechanism (Tang-Martinez, 2001).

Recognition by “association/familiarity” and by ”phenotype matching” have become the two most widely accepted mechanisms of kin discrimination (Holmes & Sherman, 1983). Whereas for recognition by association a period of previous contact (e.g. weaning) is necessary, in phenotype matching kin recognition occurs even in the absence of any previous, direct association. It has been hypothesized that the discriminator learns certain cues either from the phenotypes of close relatives (familial imprinting) (see Sherman et al., 1997) or from its own phenotype (self-matching) (Mateo & Johnston, 2000) to form a template for comparison with the phenotype of other individuals.

“Recognition alleles” are probably the most problematic of all the proposed kin recognition mechanisms. The hypothesis underlying this mechanism is that the same gene or set of genes is responsible for the phenotypic expression, for its recognition and the resulting behavior. But the present lack of direct supporting evidence suggests that this hypothesis should not be considered a high-priority contender for a kin recognition mechanism (Tang-Martinez, 2001).

Cues used for kin recognition among vertebrates may be mediated by any of the usual sensory modalities used in communication (i.e. vision, hearing, olfaction, taste and touch). Among small rodents olfactory cues are the sole or most important cues used for kin recognition. Induced anosmia experiments (Porter et al., 1978; Holmes, 1984) and the prevention of visual and auditory cues (Hepper, 1983) have led to the loss of kin recognition.

It has been hypothesized that the mechanisms involved in kin recognition might to vary with the social system of the species (Blaustein et al., 1987; Wilson, 1987; Sherman et al., 1997). In species living in stable social groups there is usually a close correlation between the degree of association of infants with different individuals and their relatedness to them. Familiarity then provides a good rule of thumb to determine relatedness as shown in Arctic ground squirrels (*Spermophilus parryii*) (Holmes & Sherman, 1982), rats (*Rattus norvegicus*) (Wills et al., 1983) and naked mole-rats (*Heterocephalus glaber*) (Clarke & Faulkes, 1999). In deer mice (*Peromyscus maniculatus*), littermates raised either together or cross-fostered and raised with non-relatives, have a reduced association and breeding between familiar compared to unfamiliar individuals, regardless of true kinship (Dewsbury, 1988). Studies of highly social species, including humans (Shepher, 1971), wild horses *(Equus cabalus)* (Berger & Cunningham, 1987), black-tailed prairie dogs (*Cynomys ludovicianus*) (Foltz & Hoogland, 1983), and naked-mole rats (*Heterocephalus glaber*) (Clarke & Faulkes, 1999) suggest that associations at early ages influence subsequent social behavior including mating behavior.

In less social species, however, it can be assumed that avoidance of kin as mates can not be based on familiarity and should be based on discrimination of degrees of relatedness (see Mateo & Johnston, 2000) using phenotype matching or self-matching. The latter should be favored in species with multiple paternity or maternity or when individuals commonly encounter older or younger siblings after dispersal (Sherman, 1991).

Social behavior

In addition to its importance in inbreeding avoidance the ability to recognize kin is also important in other kinds of social interactions, including cooperative behavior, helping and cooperative breeding.

Cooperative interactions in which individuals apparently dispense benefits to others, often at a cost to themselves, seem antithetical to evolution by natural selection, which is based on individual advantage. The often-observed behavior of helping other individuals such as the alarm call in prairie dogs when an enemy is discovered (*Cynomys*) (Hoogland, 1981) was for a long time explained by group selection. Subsequently George Williams (Williams, 1966) demonstrated that group selection is generally a weaker process than individual selection, refocusing research into the individual helping effort. Hamilton (Hamilton, 1964) introduced the theory of “altruism” based on interactions among related individuals. He argued that individuals can pass copies of their genes on the future generations not only through their own reproduction (direct fitness) but also by assisting the reproduction of close relatives (indirect fitness). “Hamilton’s rule” states that an altruistic trait can increase in frequency if the benefit (*b*) received by the donor’s relatives, weighted by their relationship (*r*) to the donor, exceeds the cost (*c*) of the action to the donor’s fitness. That is, altruism spreads if:

$rb > c$.

The more distantly related the beneficiaries are to the donor, the greater the benefit or the lower the cost to them must be for an altruistic behavior to spread. The rule specifies the conditions under which reproductive altruism evolves and there is good evidence, especially from insects and social vertebrates, that kinship provides the key to understanding altruistic behavior.

Helpers may benefit by gaining protection and feeding benefits in the established territory (Gaston, 1978; Taborsky, 1984; Taborsky, 1985), by increasing the probability of mate or territory acquisition as has been shown in the pied kingfisher (*Ceryle rudis*) (Reyer, 1980) and in a social paper wasp (*Polistes dominulus*) (Queller et al., 2000), and by increasing the probability of their survival through group augmentation (Taborsky, 1984; Brown, 1987).

The cooperatively breeding cichlid fish *Neolamprologus pulcher* must pay with help in order to be allowed to remain protected in the family group, and may eventually inherit this natal territory (Balshine-Earn et al., 1998). In Columbian ground squirrels, the daughters inherit maternal nest sites (Harris & Murie, 1984) which may help them become more easily established and reproduce successfully.

Not only territory inheritance but also rank inheritance could be an important criterion in the decision to stay. Many species of Cercopithecines (Primates) exhibit a matrilineal dominance system in which a female inherits her mother’s rank (or acquires her genealogical rank) (de Waal, 1977; Walters, 1980; Datta, 1983; Horrocks & Hunte, 1983; Chapais, 1988).

The wood mice (*Apodemus sylvaticus* Linnaeus 1758)

The wood mouse (*Apodemus sylvaticus*) is a typical European muroid rodent. *Apodemus* is a sister taxa to *Mus* and *Rattus*, the classical laboratory animals on which considerable work on social behavior has been done within the past decades (Robitaille & Bovet, 1976; Elwood, 1985; Brown, 1986; Hurst, 1987; König & Markl, 1987; Gerlach, 1996; Gerlach, 1998). Although the wood mouse has also long been the focus of investigations, most earlier studies dealt with the population structure and population dynamics dependent on different habitats and environmental conditions (Douglass et al., 1992; Gorman & Zubaid, 1993; Halle, 1993; Wilson et al., 1993b; Canova et al., 1994; Fernandez et al., 1996; Musolf, 1996; Montgomery et al., 1997; Díaz et al., 1999; Flowerdew & Ellwood, 2001). Social behaviors have only been recorded in connection with dispersal and survival. Both factors influencing the population structure and probably are determined by aggressive interactions between individuals. Recently additional social behaviors have become of interest including cooperative breeding in wood mice females (Gerlach & Bartmann, 2002).

Wood mice are considered semi-social. They can be found both alone or in groups (i.e. mother-offspring, over wintering aggregations), but associations are always temporary. Males and females have their own home range with the male’s home range being approximately 2–3 times larger than the female’s (Crawley, 1969; Tew & Macdonald, 1994). While both sexes have overlapping home ranges, home range overlap is significantly greater between males, and between males and females, than it is between females (Wilson et al., 1992; Tew & Macdonald, 1994). Observed distribution of movement patterns suggest that a single male has access to several females and as well the female can interact with several males (Tew & Macdonald, 1994). Therefore the expected mating system should be promiscuous and molecular techniques have demonstrated both in the wild (Baker et al., 1999) and in an enclosure experiment (Bartmann & Gerlach, 2001) that females mate and have offspring with more than one male at a time. In the enclosure experiment 88% of all litters was sired by up to four different males (Bartmann & Gerlach, 2001).

Wood mice are able to distinguish between individual conspecifics via urine and faeces as odor cues (Wolton, 1984) but it is not known if they can also discriminate kin from non-kin. Familiarity alone as recognition mechanism seems not to be sufficient enough to recognize all kin. Male wood mice neither cohabitate with mates nor defend territories (Brown, 1969; Wolton, 1985; Benhamou, 1990; Bartmann & Gerlach, 2001). Concurrent studies of the genetic structure and spatial distribution of wild-living wood mice showed that family groups with the exception of mothers and their unweaned pups do not exist and that dispersal distances of subadults are, on average, in a range of a few hundred meters (Gerlach, in prep.). Juvenile dispersal begins shortly after weaning (Halle, 1988; Gerlach, in prep.).

Against this background I draw the following hypothesis: wood mice should use phenotype matching than familiarity to differentiate between individuals of different genetic relatedness. In Chapter 1 of this thesis I conducted odor choice experiments to clarify the wood mice ability and the mechanism of kin recognition in female mate choice decisions. Regardless of the mechanism used I expected females to avoid inbreeding.

Male wood mice make no contribution to parenting besides their sperm (Montgomery & Gurnell, 1985; Tew & Macdonald, 1994). Therefore female mate choice should not rely on paternal care or territory defense but simply on male quality criteria. One possible male quality trait was already suggested by previous results of an enclosure study on wood mice by Bartmann & Gerlach (Bartmann & Gerlach, 2001). Here heavier males sired more offspring. If these results are caused by a preference for heavier males or by the effect of heavier males being dominant and getting more access to females through winning male-male competitions is still questioned. In Chapter 2 of this thesis I tested the influence of different male characteristics on female preference. I expect that females choose a high quality male and quality is determined by weight or dominance rank.

As an additional criterion I investigated in Chapter 3 of this thesis the influence of MHC genotype on female mate choice. Assuming the operation of pathogen-driven MHC heterozygote advantage and/or inbreeding avoidance because due to kin sharing the same alleles, MHC-disassortative mating has been predicted.

Wood mice are promiscuous and a high degree of multiple mating occurs. Males can generally increase their fitness by mating with many mates, but females cannot produce more offspring than the number of their eggs. In species without any male-provided resource benefits, females are thought to obtain some ‘genetic’ or indirect benefits from males that enhance offspring quality. Polyandry is often assumed to be a strategy to increase fertility of the mother (Hoogland, 1998), ensuring fertilization because some males are sterile (Sheldon, 1994), avoiding genetic incompatibility (Zeh & Zeh, 1996), increasing the genetic variability of offspring (Tooby, 1982), increasing litter size (Hoogland, 1998; Pearse et al., 2002), producing offspring of higher quality, increasing offspring survival (Madsen et al., 1992; Olsson et al., 1994) or avoiding inbreeding (Hosken & Blanckenhorn, 1999; Ratkiewicz & Borkowska, 2000). However, females may also mate multiply to confuse the issue of paternity in order to avoid infanticide by males.

Infanticide

Infanticide is defined as the killing of conspecific young. It is considered as an adaptive behavioral strategy to enhance the reproductive success of the perpetrator in response to intense competition for resources such as mating partners, food, or nest sites, and has been documented in many organisms, including mammals (Hausfater & Hrdy, 1984).

Laboratory studies on rodents indicate that the likelihood of an individual being infanticidal depends upon sex, reproductive status and recent sexual experience (Elwood, 1977; Soroker & Terkel, 1988; Elwood & Kennedy, 1991). The effect of recent sexual experience varies according to gender. Males are frequently inhibited from infanticide within a few days of copulation. Females, however, often continue to be infanticidal up to parturition (Elwood, 1977; Soroker & Terkel, 1988; Elwood & Kennedy, 1991).

Infanticide may be an extreme product of sexual selection in males, a way of bringing females quickly into estrous again, and thereby accelerating the infanticidal male’s own opportunities to mate (Trivers, 1972). Males may also benefit by reducing the reproductive success of competitors through infanticide, thereby causing a relative increase in their own reproductive success (vom Saal & Howard, 1982).

Since females lose much expended energy and time when infanticide occurs, attention has been focused on how females counteract infanticide by males (Hrdy, 1979; Packer & Pusey, 1983; Hausfater, 1984; Agrell et al., 1998).

In addition to the above-mentioned strategy to mate promiscuously with several (all) males in the vicinity, apparently to confuse paternity (O'Connell & Cowlishaw, 1994; Wrangham, 1997) another way for females to reduce the risk of infanticide by foreign males is to mate with a dominant male. Dominant males of many small mammal species are generally more infanticidal against offspring they have not sired (Huck et al., 1982; vom Saal & Howard, 1982), have an increased potential to attack and evict intruders (Reich et al., 1982), and exhibit higher movement activity (Nelson, 1994), than do subordinate males. Female choice of dominant males seems to be common in small rodents (Huck & Banks, 1982; Drickamer, 1992; Horne & Ylönen, 1996), as well as in other mammalian species such as lions (Pusey & Packer, 1994).

Nest site or territory defense would be another counter-strategy to prevent infanticide but in most mammals, males are larger than females and so females are not always effective in deterring intrusion by strange males.

I tested the hypothesis: Is infanticide reduced in a polyandrous system when wood mouse females mate multiple times? Results are shown in Chapter 4 of this thesis.

Paternities of these litters were assigned with microsatellite analyses. Microsatellite markers, small and highly variable fragments of non-coding DNA, have become a powerful tool for the investigation of genetic structure of populations. Because of the reduced requirements for tissue or blood to receive sufficient amounts of DNA animals can be sampled non-destructively. In my studies a small piece (2–5 mm^2) of the tissue of the ear was necessary. Even juvenile individuals could be sampled without significant perturbation. Although three microsatellite primer sets exist for the yellow-necked mouse (*Apodemus flavicollis*), which also amplify in wood mice (Gockel et al., 1997) additional special wood mouse primers are needed to accurately assess and monitor genetic variation. In Chapter 5 of my study I describe the identification of highly variable microsatellite markers in wood mice, which I then have used for paternity analyses determined in Chapter 4 and 6.

In addition to these laboratory experiments, I have studied a small population under almost natural field condition in an enclosure, large enough to prevent cage artifacts but still clear to record all animals.

In mammals females tend to remain at or near their natal site (Greenwood, 1980). Philopatry should lead to an increase in relatedness between neighboring breeding females if young females become sexually mature near their mothers and sisters. A correlation between geographic distance and relatedness has been shown in meadow voles (*Microtus pennsylvanicus*) (Sheridan & Tamarin, 1986). Matrilines, groups of related females, have been found in Richardson's ground squirrels (*Spermophilus richardsonii*) (van Staaden et al., 1994), yellow-bellied marmots (*Marmota flaviventris*) (Armitage & Schwartz, 2000), and gray-sided voles (*Clethrionomys rufocanus*) (Ishibashi et al., 1997). In some species matrilines are just found at certain times in the year (McShea & Madison, 1984; Kawata, 1990; Pusenius et al., 1998) or at certain population densities (Lambin & Krebs, 1991a). Kinship between females in bank voles (*Clethrionomys glareolus*) (Mappes et al., 1995) and Townsend’s voles (*Microtus townsendii*) (Lambin & Krebs, 1993) leads to substantial overlaps in the home ranges without decreasing the reproductive success of these females. Breeding success is higher in kin groups as well (Lambin & Krebs, 1993; Mappes et al., 1995; Solomon & French, 1997).

Telemetry studies on wood mice have revealed communal use of nest sites of reproductive females under natural conditions (Wilson et al., 1992). An enclosure experiment found communal nests with litters of two different females (Bartmann, unpublished data). Gerlach and Bartmann (2002) studied the reproductive success of breeding units of two wood mouse females with different degrees of relatedness in lab populations. In contrast to findings in the close related house mouse (*Mus musculus domesticus*) where communal breeding improved lifetime reproductive success (König, 1994) no such effect could be found in wood mice. Rather, at least one or both females paid a cost for this cooperation in terms of a reduction in the number of own offspring weaned and/or more investment by nursing the offspring of the partner. Based on these results, solitarily breeding should be preferred in wild wood mice. Under harsh environmental conditions such as low nest site availability and food limitation, other advantages support cooperative breeding. Daughters always could increase their inclusive fitness by helping their mothers to wean offspring. If cooperative breeding takes place in the wild is not known. Therefore I tested the following hypothesis in Chapter 6: Is there any incident of social behavior among wood mice females in the wild? And what benefits does a female gain by being social.

The aim of this study

Variation in female mate choice provides considerable insight into the evolution of this behavioral pattern by sexual selection (see review (Jennions & Petrie, 1997). The comparison of wood mice with other rodents such as the close related house mouse, whose social behaviors are well known, gives insight into the variability of mating systems and mate choice under different environmental conditions and social systems.

In order to investigate the evolution of mate choice decisions in wood mice I used 6 different approaches:

(1) Experiments on kin recognition and mate choice under laboratory conditions

(2) Experiments on mate preferences under laboratory conditions

(3) Investigation on the influence of MHC genotype on female mate choice in the lab and in the enclosure

(4) Laboratory experiments to determine the causes of multiple mating in female wood mice

(5) Development of DNA microsatellite markers for the analysis of paternity and reproductive success

(6) Experiments to study the impact of matrilinear groups on reproductive success of females under semi-natural conditions in a large enclosure

This study, combining the advantages of both laboratory and field approaches, presents new data on mating behavior in the wood mouse. Insights gained from these studies of mate and helper choice in wood mice provide a basis for future studies of mating behavior in other species with reduced sociality. Studies on the evolution of social behavior and its fitness consequences contribute to our understanding of different social systems under various ecological conditions.

CHAPTER 1: KIN RECOGNITION AND INBREEDING AVOIDANCE BY PHENOTYPE MATCHING IN FEMALE WOOD MICE (*APODEMUS SYLVATICUS*)

Keywords *Apodemus* · Kin recognition · Female mate choice · Inbreeding avoidance

Submitted to *Behavioral Ecology and Sociobiology*

Abstract

It is assumed that mechanisms for recognizing kin and avoiding inbreeding are based on familiarity in more social species and on phenotype matching in less social species because the latter do not grow up in family groups. We tested this prediction in the semi-social wood mouse (*Apodemus sylvaticus*) where we would expect phenotype matching. In preference tests for male odor we show that indeed females in estrus, but not outside their estrus period, avoided and thus recognized the odor of unfamiliar brothers in comparison with unfamiliar unrelated males. While this result corresponds to prediction, careful comparison with previous studies on kin recognition in social and less social rodents shows that sociality *per se* cannot be used as a predictor for the mechanism involved in inbreeding avoidance.

Introduction

The avoidance of close kin as possible mating partners is assumed to be under strong selection pressure as inbreeding decreases fertility and survival of offspring (Ralls et al., 1986; Charlesworth & Charlesworth, 1987). While avoidance of close kin as mating partners has been shown in several species (Wilmsen Thornhill, 1993) the mechanisms individuals use for differentiating kin from non-kin are less clear. ‘Indirect’ recognition involves cues that are predictably associated with kin (e.g. location of a nest), whereas ‘direct’ recognition refers to perception and evaluation of kin cues (e.g. their odors) (Waldman et al., 1988; Barnard, 1990). There are two general categories of kin recognition mechanisms both based on learning processes as Tang-Martinez (2001) emphasized. The first 'direct' mechanism is based on aspects of familiarity where individuals behaved nepotistic to conspecifics with whom they grow up. The ability to identify even unfamiliar kin is usually attributed to the second 'direct' kin recognition mechanism of 'phenotype matching' in which an individual must learn cues either from the phenotypes of close relatives (familial imprinting) (see Sherman et al., 1997) or from its own phenotype (self-matching) (Mateo & Johnston, 2000) to form a template for comparison with the phenotype of other individuals. It has been hypothesized that the mechanisms involved might to vary with the social system of the species (Blaustein et al., 1987; Wilson, 1987;

Sherman et al., 1997). In species living in stable social groups there is usually a close correlation between the degree of association of infants with different individuals and their relatedness to them. Familiarity then provides a good rule of thumb to determine relatedness as shown in Arctic ground squirrels (*Spermophilus parryii*) (Holmes & Sherman, 1982), rats (*Rattus norvegicus*) (Wills et al., 1983) and naked mole-rats (*Heterocephalus glaber*) (Clarke & Faulkes, 1999). In deer mice (*Peromyscus maniculatus*), when littermates were either raised together or cross-fostered and raised with non-relatives, association and breeding between familiar compared to unfamiliar individuals was reduced, regardless of true kinship (Dewsbury, 1988). Studies of highly social species, including humans (Shepher, 1971), wild horses *(Equus cabalus)* (Berger & Cunningham, 1987), black-tailed prairie dogs (*Cynomys ludovicianus*) (Foltz & Hoogland, 1983), and naked-mole rats (*Heterocephalus glaber*) (Clarke & Faulkes, 1999) suggest that associations at early ages influence subsequent social behavior including mating behavior.

In less social species, however, it can be assumed that avoidance of kin as mates can not be based on familiarity and should be based on discrimination of degrees of relatedness (see Mateo & Johnston, 2000) using phenotype matching or self-matching. The latter should be favored in species with multiple paternity or maternity or when individuals commonly encounter older or younger siblings after dispersal (Sherman, 1991).

We tested this prediction in a semi-social species with a high degree of promiscuity (Baker et al., 1999; Bartmann & Gerlach, 2001), the wood mouse (*Apodemus sylvaticus*), where females can be expected to use phenotype matching to differentiate between males of different genetic relatedness. In wood mice familiarity alone would not be sufficient to recognize all kin. Male wood mice neither cohabitat with mates nor defend territories (Brown, 1969; Wolton, 1985; Benhamou, 1990; Bartmann & Gerlach, 2001). Male wood mice make no contribution to parenting besides their sperm (Montgomery & Gurnell, 1985). Concurrent studies of the genetic structure and spatial distribution of wild-living wood mice showed that family groups with the exception of mothers and their un-weaned pups do not exist and that dispersal distances of subadults are, on average, in a range of a few hundred meters (Gerlach unpub. results). Juvenile dispersal begins shortly after weaning (Halle, 1988; Gerlach unpub. results). Therefore, female wood mice can encounter both related and unrelated males as potential mating partners. While individual recognition exists in wood mice (Wolton, 1984), the mechanisms involved in kin recognition remain unknown.

We tested first if female mate choice behavior depended on their estrus cycle. Secondly, we examined if preferences of females are based on effects of familiarity or relatedness. Since preferences for olfactory cues are associated with mate choice (Krackow & Matuschak, 1991; Pillay, 2000), we tested female preference for urine of different males.

Methods

Experimental animals were the F1–F2 generation of wild caught wood mice from different places in southern Germany (Konstanz and Reutlingen); they were assumed to be unrelated. For breeding purposes mice were kept in monogamous pairs in two cages (42 x 26 x 15 cm) and a nest box (14 x 14 x 14 cm) linked by plastic tubes. All animals were kept at 20°C under a long photoperiod (14L:10D; light on at 8:00 am). Food pellets (Altromin 1310) and water were offered *ad libitum*. Offspring were removed from the parental cage system when they were 28 days old. Offspring were kept together until the experiments were conducted when they were approximately 8 weeks old. Wood mice reach sexually maturity with the age of approximately 8 weeks. We found no evidence of delayed maturation caused by keeping siblings in family groups because all individuals immediately reproduced when put together with an unfamiliar partner regardless of their own age (personal observation).

Vaginal smears of the test female taken 5 hours before the odor test indicated her reproductive stage. Vaginal smears were obtained by carefully stroking a plastic eyelet moistened with warm water along the vagina wall of the female. The cell material was transferred to a microscope slide, dried at 60°C for at least 30 min and then stained with HEMA-DIFF reagents (bioanalytic, Umkirch/Freiburg, Germany, Art.Nr. 4100). The vaginal smear was examined under a light microscope (100x magnification). The most common cell types observed were nucleated or cornified (old, non-nucleated) epithelial cells and leukocytes. Every stage of the estrus cycle (met-estrus, di-estrus, pro-estrus, estrus) is defined by the percentage of these cell types (Snell, 1941; Flowerdew, 1987). In wood mice three types of estrus cycles were identified: females with a continuous estrus; females with an estrus cycle between 6 and 11 days in length; and females that only rarely showed signs of estrus (Jonsson & Silverin, 1997).

Odor preferences of individual female mice were tested in a Plexiglas Y-maze preference apparatus (5 cm diameter) with 30 cm long arms (see Fig. 1 for illustration). The stimulus compartments in the two arms of the Y in which odor cues were placed, and the start box, in which a mouse was placed, were each 14 cm long. A solid plexiglas barrier restricted the introduced mouse to the start box, while perforated plexiglas barriers at the end of each stimulus arm prevented direct contact with the odor sources. Small fans fitted at the end of the stimulus compartments produced a low air current towards the start box. Single males were placed for 2 hours in small boxes with bedding material, which was used as an odor source. To avoid side preferences we alternated the side with odor from males of different relatedness and familiarity between trials. The preference apparatus was washed thoroughly between trials.

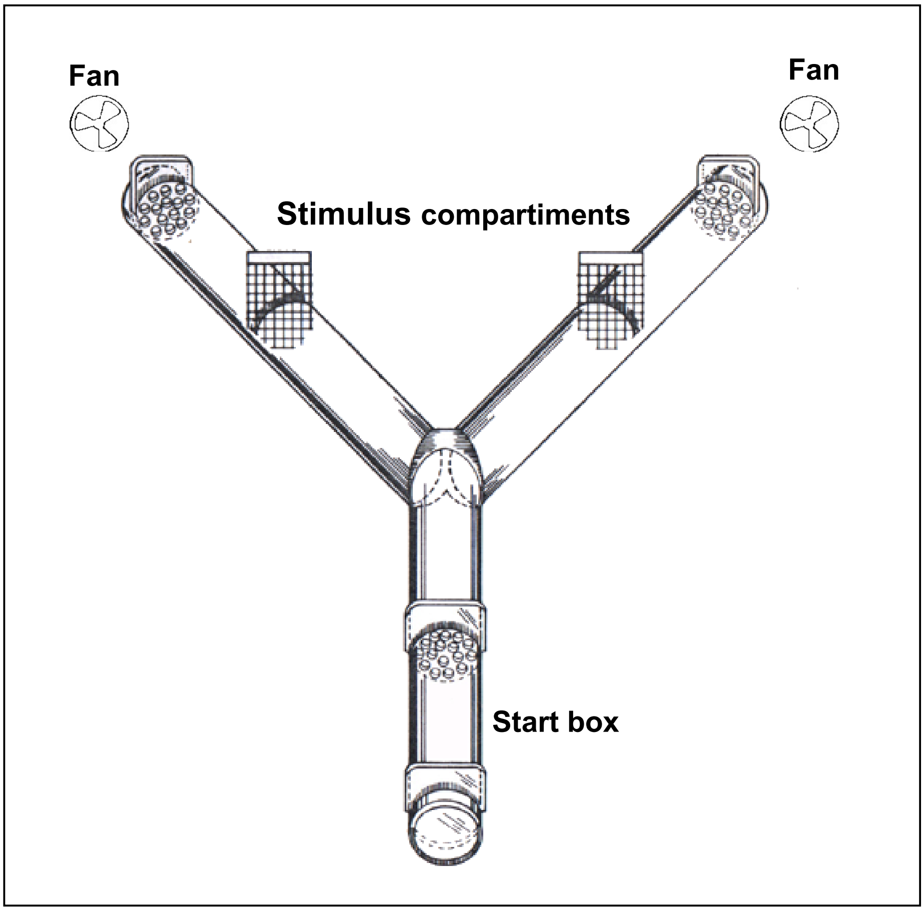

Fig. 1. Y-maze odor preferences apparatus

After a female mouse was placed in the start box of the apparatus for 5 min the solid barrier was removed allowing the mouse access to the two arms of the Y-maze with bedding material of different males. During the subsequent 10 min the behavior of the mouse was videotaped and the duration of the time spent in each arm of an odor source was recorded. Videotapes were analyzed blind as to the identity of the odor donors. 'Preference' was defined as the amount of the time that the test female spent in one stimulus arm divided by the amount of time spent in the other stimulus arm. All statistical tests were based on an analysis of the natural logarithm of this ratio (Coopersmith & Lenington, 1992). Individual trials were discarded if a female spent less than 30 s of the 10 min test in the distal end of each of the stimulus arms (Kavaliers et al., 1997).

Data were analyzed by using the program JMP (SAS Institute Inc., 2001) and non-parametric methods according to Siegel (1997).

In experiment 1 females of all stages of the estrus cycle were tested. All females were just tested once. Two odor cues, one of a familiar related male (FR, a familiar brother and littermate of the female) and an unfamiliar unrelated male (UFUR) were placed in the Y-maze. Females were only separated from their familiar brothers for the 2 hours the male mice were put individually on bedding to get odor cues for the test. For the second experiment only estrous females were used. In experiment 2 an unfamiliar related male (UFR, unknown brother from a former litter who had had no previous contact with the female) and an unfamiliar unrelated male (UFUR) were used as stimulus animals. All male test pairs were matched for age.

Results

Female wood mice showed different preferences for male odor cues depending on their estrus cycle. 18 females were tested for their preference for the odor cues of familiar related (FR) versus unfamiliar unrelated males (UFUR). Data of females that were in met-estrus (n = 2), di-estrus (n = 4) and pro-estrus (n = 3) were pooled as non-estrus and tested against females in estrus (n=13). In an analysis of variance 'estrus stage' was used as the independent variable, 'preference' as the dependent variable. 'Estrus stage' had a significant influence on female preference for males (ANOVA: F = 5.082, df = 1, P = 0.036). In addition, there was a trend for females in estrus to spend more time with the odor cue of an unfamiliar, unrelated male (UFUR) than with a familiar related male (FR) (mean time [s] ± SE with UFUR = 117.5 ± 45.6; mean time with FR = 45.6 ± 12.1; Wilcoxon signed-rank test, n = 13, T = 1.63, P = 0.057, two-tailed), and for non-estrous females to spend more time with the odor of their familiar brother. However, this difference was statistically not significant (mean time [s] ±SE with UFUR = 47.1±8.6; mean time with FR = 122.3 ± 47.8; Wilcoxon signed-rank test; n = 9, T = 1.83, P = 0.10, two tailed) (Fig. 2).

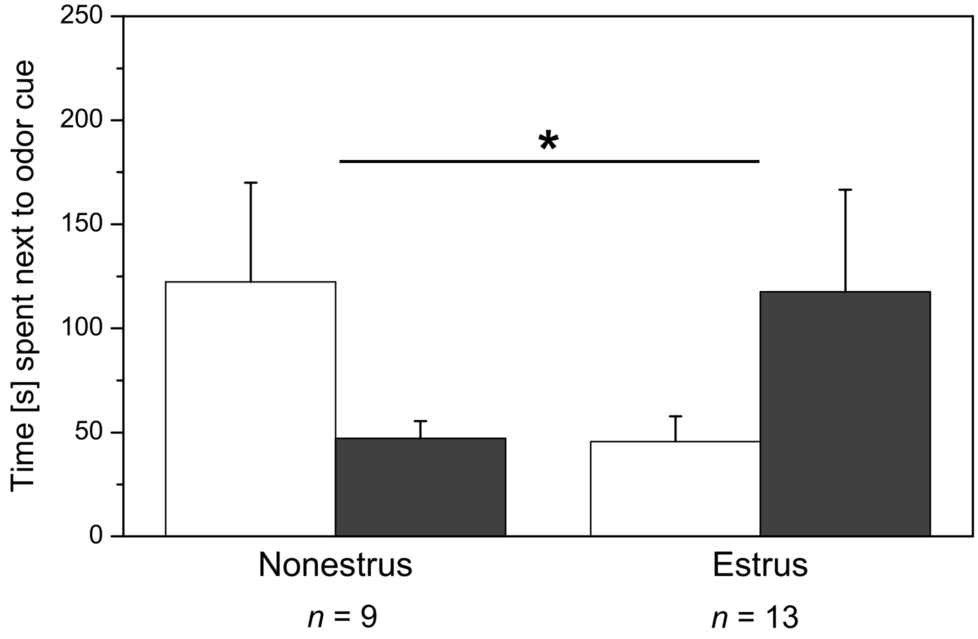

Fig. 2. Mean duration ([s] + standard error, SE) female wood mice spent next to the odor cue of a familiar related male = FR (white box) and an unfamiliar unrelated male = UFUR (black box) within an observational period of 10 minutes; the estrus stage has a significant influence on the females' preference.

To evaluate whether previous experience and cohabitation with a male determined female choice we used odor cues of unfamiliar brothers (UFR) versus those of unfamiliar unrelated males (UFUR). Only females in estrus were used (*n* = 10). Females spent significantly more time with the odor of the unfamiliar unrelated male than with that the odor of the unfamiliar related male (mean time [s] ±SE with UFUR = 148.1 ± 26.7; mean time with UFR = 62.5 ± 5.6, Wilcoxon signed-rank test: *n* = 10, *T* = 2.79, *P* = 0.038, two tailed) (Fig.3).

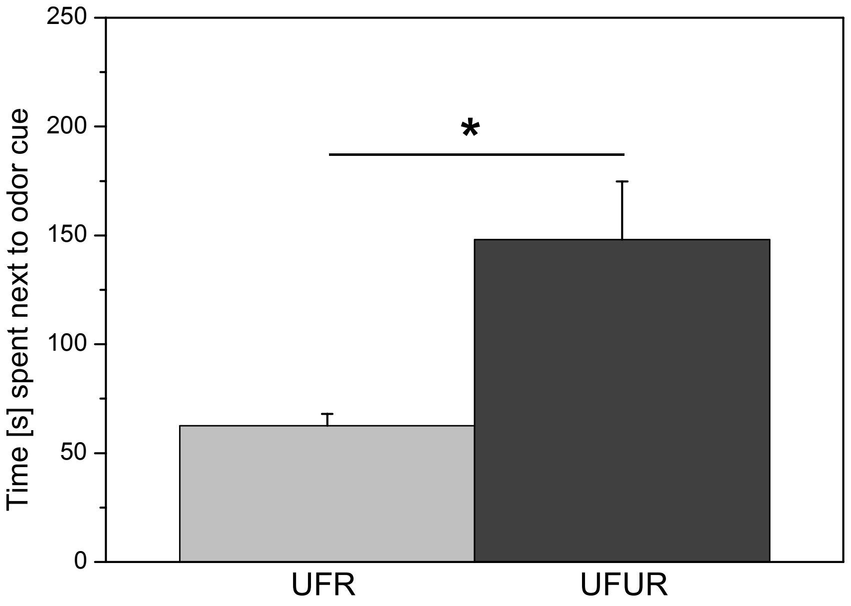

Fig. 3. Mean duration ([s] + standard error, SE) estrous female wood mice spent next to the odor cue of an unfamiliar related male = UFR (gray box) and an unfamiliar unrelated male = UFUR (black box); females significantly preferred the unrelated male; sample size *n* = 10.

Therefore, we conclude that female wood mice were able to recognize even unfamiliar kin.

Discussion

Estrous female wood mice preferred the urine odor of unfamiliar unrelated males to the odor of familiar brothers; they did not show this preference while being in a non-estrus stage. Whereas the preference for a specific male was not but almost significant ($P = 0.057$ / $P = 0.10$), the difference in behavior of estrus and non-estrus females was. So females actually showed a different behavioral pattern when they were in mating conditions. Social preferences like the association with familiar individuals as have been found for non-estrus females could be distinguished from sexual preferences (avoidance of familiar individuals) expressed by females in estrus. In several rodent species preferences for familiar siblings in social interactions have been found (e.g. *Peromyscus leucopus* (Halpin & Hoffman, 1987); *Acomys cahirinus* (Porter et al., 1983); *Spermophilus parryii* and *S. beldingi* (Holmes & Sherman, 1982)). Maybe in our first experiment still existing social preferences of estrous wood mouse females hinder the results from becoming significant. Wild female house mice (*Mus musculus*) when given the choice between a familiar brother and an unfamiliar brother spent more time with the familiar male (Winn & Vestal, 1986), although in pairs with unrelated males, always the unrelated males were preferred.

The most striking result from our experiment is that familiarity with the male was not the crucial factor in these tests of odor preference: females also preferred odors of unfamiliar unrelated males to the odor of brothers with whom they had no previous contact. This shows that they could recognize unfamiliar kin using phenotype matching. From this odor preference we would predict that given a choice females should avoid mating with brothers and perhaps other related individuals. That some kind of inbreeding barrier or inbreeding avoidance exists between siblings was shown additionally by the fact that littermates, when kept together after weaning, reproduced just in 5–10% of all cases whereas almost 100% of unfamiliar unrelated couples bred (Musolf, personal observation).

This recognition can be based on comparing the odor of novel males with either their own phenotypic odor template or that of familial conspecifics (littermates or mothers). This template could be formed by cues from the major histocompatibility complex (MHC) gene products (Apanius et al., 1997; Jordan & Bruford, 1998). MHC class I and II antigens are secreted in soluble form into the blood and are excreted in a degraded form in the urine. This process gives rise to volatile components, which are unique to each individual but more similar in related animals (Brown et al., 1989).

In our study we could not differentiate whether females used self-matching or phenotype matching based on templates of littermates or of their mother. The ability to self-match is controversial (see (Alexander, 1991; Sherman et al., 1997). In the non-social, promiscuous golden hamster (*Mesocricetus auratus*) self-matching might be used in mate choice decisions (Mateo & Johnston, 2000). Considering the high degree of multiply sired litters and the promiscuous mating system in wood mice (Baker et al., 1999; Bartmann & Gerlach, 2001) self-matching would also be an appropriate mechanism for differentiation between individuals of different genetic relatedness in wood mice.

Using genetically based templates is assumed to be especially useful in those species that do not live in social systems where familiarity can provide sufficient information to avoid breeding with close relatives (Blaustein et al., 1987; Wilson, 1987; Sherman et al., 1997). To evaluate if sociality of a species plays a role in determining the mechanism involved in avoiding mating with relatives, we compare our results with those of previous studies on female mate choice in rodents.

The role of familiarity versus phenotype matching can be determined by odor choice tests or by mate choice tests asking females to differentiate between familiar/unfamiliar and related/unrelated odors or individuals. Female mate choice in highly social house mice showed that females differentiated between related and unrelated individuals but the role of familiarity could not be ruled out (Gilder & Slater, 1978; Egid & Lenington, 1985; Lenington & Egid, 1985; Winn & Vestal, 1986; Dewsbury, 1988; Potts et al., 1991). Yamazaki et al. (1988) showed that inbred male house mice typically prefer to mate with females of a different, non-self H-2 haplotype. This mating preference is acquired by imprinting on familial H-2 types indicating that familiarity is also important. In two additional studies on mate choice in house mice no discrimination was found (Barnard & Fitzsimons, 1988; Krackow & Matuschak, 1991). However, Barnard and FitzSimons (1988) did not evaluate the estrus stage of female mice and Krackow and Matuschak (1991) tested unfamiliar unrelated males against unfamiliar half-brothers, which might influence preferences. Our results show the confounding effect of estrus: only estrous females avoided kin odor whereas non-estrous females do not and may even prefer to associate with kin. Half brothers may be avoided less than more closely related full brothers and fathers. In the social deer mouse (*Peromyscus maniculatus*) Hill (1974) showed that early exposure to males inhibited onset of reproduction indicating that familiarity might play a role in reproductive physiology. In the white-footed mouse (*Peromyscus leucopus*) estrous females differentiated on the basis of genetic relatedness, but here familiarity was not tested (Grau, 1982; Keane, 1990). In three social *Microtus* species, the prairie vole (*M. ochrogaster*) (Gavish et al., 1984; DeVries et al., 1997) the gray tailed vole (*M. canicaudus*) (Boyd & Blaustein, 1985) and the mandarin vole (*M. mandarinus)* (Fadao et al., 2000), familiarity was shown to be used as a mechanism in mate choice, but only in *M. mandarinus* and *M. canicaudus* was it proven that genetic relatedness played no role (Fadao et al., 2000; Boyd & Blaustein, 1985). In social gerbils (*Meriones unguiculatus*) (Ågren, 1984) females did not differentiate between unfamiliar related or unfamiliar unrelated males indicating that phenotype matching was not used but the role of familiarity remained unclear. In the highly social naked mole-rat (*Heterocephalus glaber*) (Clarke & Faulkes, 1999), familiarity but not relatedness determined mate choice. In summary, in social rodents 2 species were shown to use phenotype matching while in 5 others differentiation was based on familiarity. Exclusive use of familiarity could be shown in 4 species; only one species was shown to use both mechanisms.

Fewer studies are available for less social species. In the golden hamster (*Mesocricetus auratus*) (Mateo & Johnston, 2000) and in the cactus mouse (*Peromyscus eremicus*) (Dewsbury, 1982a) females used both phenotype matching and familiarity, while meadow vole females (*Microtus pennsylvanicus*) (Salo & Dewsbury, 1995) were shown to use familiarity while the role of relatedness was not studied. Our results now show that female wood mice use phenotype matching. Therefore, both mechanisms can be used in less social rodent species.

There is a trend that social rodents more often use familiarity and not phenotype matching while both mechanisms seem to be used in less social species. This contradicts the assumption that phenotype matching should be more important in less social species. Obviously, sociality of a species *per se* does not allow a good prediction for which kin recognition mechanism is used in mate choice decisions. Other factors should be considered to explain the evolution of kin recognition in rodents. A phylogenetic analysis of these rodent species would be useful to explain the emergence of kin recognition systems.

Acknowledgement We wish to express our gratitude to Jelle Atema and Kirsten Pohlmann for valuable criticism of the manuscript and W. Nagl for statistical advice. We also thank R. Hellmann for laboratory help and H. Markl for generously supporting the project. This study was supported by the University of Konstanz, 'Verband der Chemischen Industrie' and by the Deutsche Forschungsgemeinschaft DFG (Ge 842/1-3). All experiments comply with the current laws of Germany.

CHAPTER 2: FEMALE WOOD MICE (*APODEMUS SYLVATICUS*) ARE NEARLY INDISCRIMINATE IN CHOOSING MALES OF DIFFERENT QUALITY

Keywords *Apodemus* · Female mate choice · Male quality

Submitted to *Behavioral Ecology and Sociobiology*

Abstract

Female preference for male traits indicating dominance or good physical condition has been shown in numerous species of small mammals. Their choice is based often on urine cues. Most of the species studied are rather social with stable dominance relationships. We investigated male odor preference in female wood mice (*Apodemus sylvaticus*), a semi-social species in which females have multiple mating partners for each estrus cycle, to see if and how they evaluate their multiple partners and what information they can obtain from their urine odor. We analyzed female preference for odor cues of males of different body weight, dominance, urination pattern and age. Previous results showing that heavier male wood mice had greater reproductive success in terms of number of offspring than lighter males suggested that females should prefer heavier males. Surprisingly our study showed that females did not prefer the odor of the heavier male of a pair and also not the odor of dominant, older or more experienced males. There was also no relationship – as seen in other species – between body weight, urination pattern, and aggressive behavior in these male wood mice. Finally, female odor preference was not affected by females copying the mating behavior of other females, although females avoided odor of another female associated with a male. Our results indicate that female preference for male traits is probably less evolved in a species with a naturally promiscuous mating system with multiple matings in every estrus period. The higher reproductive success of heavier males could then be explained not as a result of female choice but with different sperm quantity or quality, or with sperm competition.

Introduction

Molecular techniques have shown in numerous species that females mate and have offspring with more than one male at a time. Despite many studies examining causes and benefits of multiple paternity its relationship with female choice and, as a consequence, sexual selection remains unclear. We wanted to analyze female preference for specific males (of higher quality) in a species with a high degree of multiple mating. Genetic determination of paternity in litters of wood mice

(*Apodemus sylvaticus*) showed that under natural conditions multiple mating occurs (Baker et al., 1999); in an enclosure experiment 88% of all litters were sired by up to four different males (Bartmann & Gerlach, 2001). Interestingly, reproductive success was not equally divided among all males. Heavier males sired significantly more offspring than lighter ones (Bartmann & Gerlach, 2001). This suggests male body weight as a possible assessment mechanism for female choice.

There are numerous suggestions as to what females can gain by being selective in their choice of mates (Kirkpatrick & Ryan, 1991; Andersson, 1994). Females can gain not only benefits such as essential territorial resources, paternal care or food gifts, but also their immediate reproductive success, fecundity and/or the quality of offspring, all of which can vary among males (Drickamer et al., 2000). In species such as wood mice, in which males do not provide parental care, females might choose mating partners whose genes will confer greater fitness (including mating success) on the female's offspring ('good genes' hypothesis) (Møller & Alatalo, 1999). Females could use any male trait correlated with fitness as a guide to advantageous matings. Differences in male quality can be assessed by among others chemical signaling, physical strength, ornaments and parasite load (Møller, 1990; Milinski & Bakker, 1990). There is ample empirical evidence for female choice based on male traits (such as the spur length of male pheasants (*Phasianus colchicus*) (von Schantz et al., 1989), the brighter coloration of male sticklebacks (*Gasterosteus aculeatus*) (Milinski & Bakker, 1990) and male house finches *(Carpodacus mexicanus)* (Hill, 1990)) that reflect vigor and recent studies indicate that females can increase the survival of their offspring by mating with a high quality male (Norris, 1993; Petrie, 1994; von Schantz et al., 1994; von Schantz et al., 1996; Hasselquist et al., 1996).

In small mammals higher body weight is indicative of dominance (Wolff, 1985b but see Gosling et al., 2000). Barnard & Fitzsimons (1989) found in laboratory mice a significant correlation between male rank and body weight with heavier males having higher rank. Also a good predictor of adult (*Mus musculus*) dominance status was the rate of scent marking: males that marked more than their brothers were more likely to become dominant (Collins et al., 1997). The urine of male mice contains major urinary proteins whose function may be to mediate the controlled release of pheromones in scent marks (Hurst et al., 1998). The quality of an individual's odor can allow potential mates to discriminate against individuals of low social status, poor health status or unsuitable genotype (reviewed by Brown & Macdonald, 1985). Competitive scent marking provides a further mechanism to allow females to discriminate between males of apparent high quality (Rich & Hurst, 1998). Finally, higher reproductive success of heavier males could be caused by females copying mate choice decisions of other females (Gibson & Höglund, 1992; Pruett-Jones, 1992).

Copying behavior has been demonstrated in several species of fish, for example the guppy (*Poecilia reticulata*) (Dugatkin & Godin, 1992), the Japanese medeka (*Oryzias latipes*) (Grant & Green, 1996), the sailfin molly (*Poecilia latipinna*) (Schlupp & Ryan, 1996) and in several lekking species of birds like the Japanese quail (*Coturnix coturnix*) (Galef & White, 1998), the sage grouse (*Centrocercus urophasianus*) (Gibson et al., 1991), the black grouse (*Tetrao tetrix*) (Höglund et al., 1995) but also in fallow deer (*Dama dama*) (Clutton-Brock et al., 1989). In all these species males do not provide parental care. Copying could serve either to increase the accuracy of mate assessment or to reduce its costs.

The aim of this study was to determine mate choice criteria of female wood mice. Since in small rodents females often use olfaction to assess males, we conducted a series of olfactory discrimination experiments. First we tested female preference for male weight characteristics. Second, we tested their preference for male dominance and urination behavior, traits generally associated with body weight. And third, we were interested if mating of one female influences the preference of another female for the same male. Finally, we determined the correlation between male body weight, dominance and urination behavior.

Methods

Experimental animals were the F1 and F2 generation of wild caught wood mice from different places in southern Germany (Konstanz, Reutlingen); they were assumed to be unrelated. For breeding purposes mice were kept in monogamous pairs in two cages (42 x 26 x 15 cm) and a nest box (14 x 14 x 14 cm) linked by plastic tubes. All animals were kept at 20°C under a long photoperiod (14L:10D; light on at 8:00 am). Food pellets (Altromin 1310) and water were offered *ad libitum*. Offspring were removed from the parental cage system when they were 28 days old. Offspring were kept together until the experiments were conducted when they were approximately 8 weeks old.

Odor preferences of individual female mice were tested in a Plexiglas Y-maze. Each arm was 5 cm diameter and 30 cm long (See Fig. 1 in Chapter 1 for illustration). Attached to the two upstream stimulus arms were 14 cm long compartments in which odor cues could be placed. Small fans upstream of the stimulus compartments produced a low air current towards the start box attached to the downstream arm in which a mouse could be placed. Perforated plexiglas barriers at the end of each stimulus arm prevented direct contact with the odor sources. A loosely fitting plexiglas barrier initially restricted the introduced mouse to the start box.

As odor sources we used bedding material from single males that had been placed for 2 hours in small boxes as describes in Kavaliers et al. (1994). During this time males normally have urinated. If not we blew at them, which immediately led to urination. Bedding was used as urine carrier because of the easy handling; paper would be often destroyed by gnawing mice. The preference apparatus was washed thoroughly between trials.

After a female mouse was placed in the start box for 5 min the barrier was removed allowing the mouse access to the two arms of the Y-maze. During the subsequent 10 min the behavior of the mouse was videotaped and the time spent in each upstream arm was recorded. ”Preference” was defined as the amount of the time a female spent in one stimulus arm investigating the odor source divided by the amount of time spent actively in the other stimulus arm. All statistical tests were based on an analysis of the natural logarithm of this ratio (Coopersmith & Lenington, 1992). Individual trials were discarded if a female spent less than 30 s of the 10 min test in the distal end of the stimulus arms (Kavaliers et al., 1997). To avoid side preferences we randomly assigned sides of the stimulus arms for each trial. Vaginal smears of the test female taken 5 hours before the odor test indicated her reproductive stage (Snell, 1941; Flowerdew, 1987). We increased the number of receptive females needed for this experiment by exposing for one week groups of sisters to the odor of an unrelated male through mesh wire barriers. This procedure induced estrus as shown in house mice (*Mus musculus*) (Whitten, 1956) and wood mice (Stopka & Macdonald, 1998). After this stimulation period almost 100% of the females were in estrus.

In experiment 1 'Body weight' we tested if females differentiate between males of different body weight. For stimulus animals we chose two brothers of the same litter so that they would not differ too much in additional traits, which we could not control (heavy male = HM; light male = LM). Each replicate was done with a new pair of males and a new female.

In experiment 2 'Dominance' we tested if females prefer dominant males. We tried to determine dominance by comparing body weight, aggressive interactions and urine marking patterns in two unfamiliar brothers. To avoid that these males had already established a dominance hierarchy during their upbringing we used unfamiliar brothers of two consecutive litters. Both males were placed overnight (16–20 h) in one cage (58 x 35 x 19 cm) separated by a mesh wire barrier, which allowed no physical contact. Filter paper at the bottom of the cage allowed us to analyze urine marking patterns for assessing dominance rank as described in (Gosling & McKay, 1990; Horne & Ylönen, 1996): 1. Dominant: fine trace urination, particularly along the partition but also in other parts of the arena. No or small and few puddles. 2. Subordinate: no fine traces. Large puddles of urine, usually deposited in the corners furthest from the partition.

The next day males were placed for 2 hours in small boxes with bedding material for use as odor sources and the preference test was conducted. Thereafter the two males were put together in a clean cage (42 x 26 x 15 cm) and during the following 10 minutes aggressive behavior was observed and dominance determined. Attacks and chasing were used as dominance criteria. The male with clearly more aggressive encounters was determined as dominant. Again, new animals were used in each replicate.

In experiment 3 'Copying' we tested if females prefer males that had already cohabited and probably mated with another female (copying behavior). For one week the test female was kept in a central cage interconnected with two additional cages: one with a solitary male and one with a male cohabiting with a female. The males were brothers of the same litter raised together. The additional female was unrelated to both test female and stimulus males. Prior to the preference test each male was placed for 2 hours in a small box with bedding material used as odor source (Series A), or bedding material of the solitary male was tested as a stimulus against bedding material of the couple (Series B). For each replicate Series A and B was done with the same animals, but each replicate was done with new animals.

All males used in these experiments were unrelated and unfamiliar to the test female. Data were analyzed using the program JMP (SAS Institute Inc., 2001) and non-parametric methods according to Siegel (1997). The calculated statistical significances are based on a two-tailed analysis.

Results

1. Do females prefer heavier males?

Among the three experimental groups there was no difference in female preference for males of different body weight (ANOVA, *df* = 2, *F* = 0.048, *P* = 0.954). Although females spent, on average, 137 s (± 13 SE) with the heavier male and 114 s (± 11 SE) with the lighter ones this difference was statistically non-significant (Wilcoxon signed rank test, *n* = 43, *T* = 1.45, *P* = 0.227) (Fig.1). The body weight of the heavier male ranged from 22.8 g to 56.7 g and that of the lighter males from 18.5 g to 50.5 g; the mean difference in body weight between the two males was 7.4 g (± 0.8 SE). Although not statistically significant female preference for the heavier male increased with increasing weight difference between males (ANOVA, *F* = 1.87, *P* = 0.179)

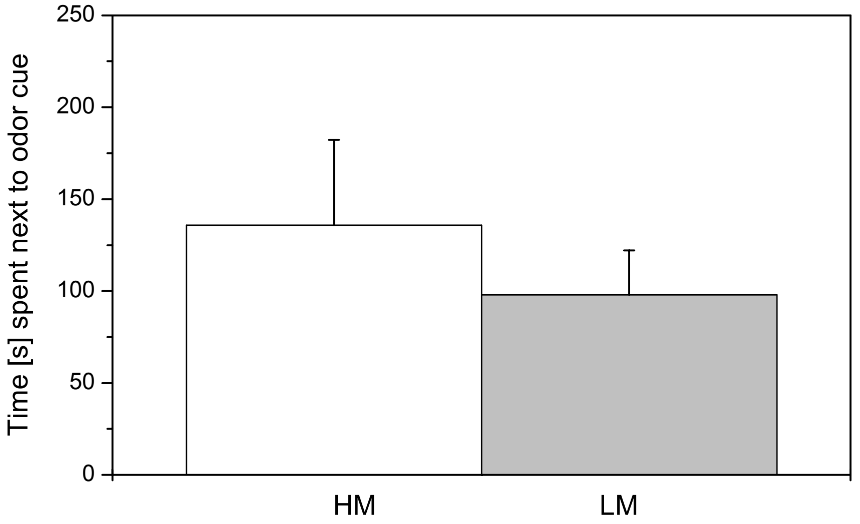

Fig. 1. Mean duration ([s] + standard error, SE) estrous female wood mice spent next to the odor cue of a heavy male = HM (white box) and a light male = LM (gray box) within an observational period of 10 minutes; no significant preference was observed; sample size *n* = 43.

2. Do females prefer males according different dominance criteria?

To analyze the relationship between body weight, urination pattern and dominance in male wood mice we conducted a second experiment with 19 test pairs of males: 15 of the pairs fought when put together and a dominant male (DM) could be determined. In 9 cases the winner was the heavier male, in 6 sets the lighter male won. Therefore, there was no significant relationship between body weight and dominance (Wilcoxon signed-rank test: $n = 15$, $T = 0.641$, $P = 0.720$).

We could also not find any indication that dominant and subordinate males showed different urination patterns (Wilcoxon signed-rank test: $n = 5$, area: $T = 0.564$, $P = 0.625$; spots: $T = 0.211$, $P = 0.313$).

Heavy and light males urinated similarly in terms of number of urine spots (4–22 spots) and covered area (mean area [cm^2] ± SE: HM = 64.2 ± 2.1, LM = 64.4 ± 2.2). All males produced at least one big puddle of urine. Although there was a difference in urination pattern between males in general (4–22 spots), paired males always behaved similarly (mean difference in area [cm^2] ± SE = 5.8 ± 0.9, mean difference in spots ± SE = 3.5 ± 0).

Females did not prefer odor of dominant males (DM) to subordinate males (SubM) (Mean time [s] ± SE with DM = 144.7 ± 20.2; mean time with subordinate male (SubM) = 121.5 ± 20.6; Wilcoxon signed-rank test: *n* = 15, *T* = 0.301, *P* = 0.303) (Fig. 2).

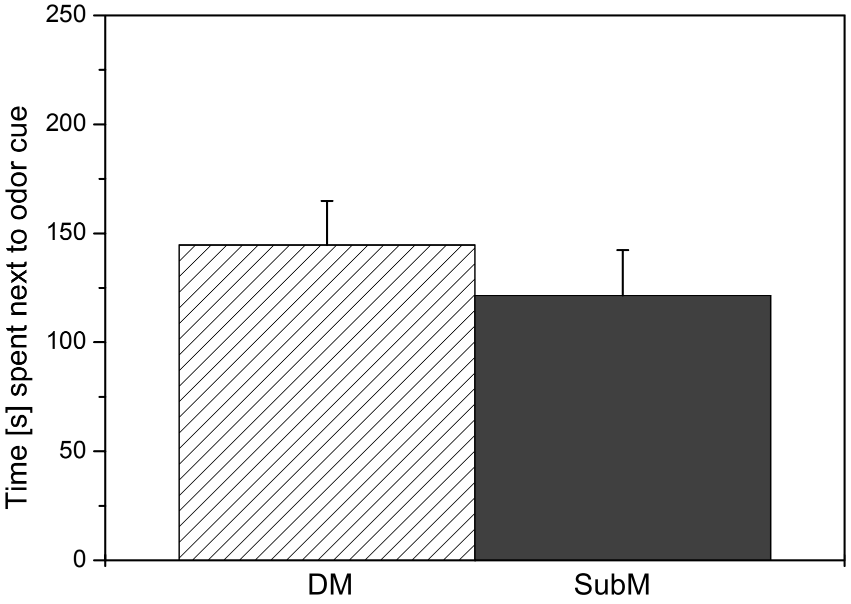

Fig. 2. Mean duration ([s] + standard error, SE) estrous female wood mice spent next to the odor cue of a dominant male = DM (striped box) and a subordinate male = SubM (black box) within an observational period of 10 minutes; no significant preference was observed; sample size *n* = 15.

Males in this experimental treatment differed in age by about 1–2 month. But the age of males did not influence female preferences either. Females spent, on average 153 s ± 20 SE with the older male and 111 s ± 12 SE with the younger male; Wilcoxon signed-rank test: *T* = 0.1039, *P* = 0.145).

3. Do females show copying behavior?

In series A we tested female preference for odor cues of solitary males compared to paired males. Females showed no preference for either the solitary male (SoM) or the paired male (PM) (Mean time with SoM = 117 s ± 15 SE; mean time with PM = 121 s ± 20 SE; Wilcoxon signed-rank test: *n* = 15; *T* = 0.881, *P* = 0.804).

In series B, females could choose between odor cues of the solitary male compared to the odor of the male and female pair. Test females significantly avoided the odor cue of the pair (Mean time with SoM = 167 s ± 16 SE; mean time with couple = 94 s ± 12 SE; Wilcoxon signed-rank test: *n* = 8, *T* = 0.021, *P* = 0.008).

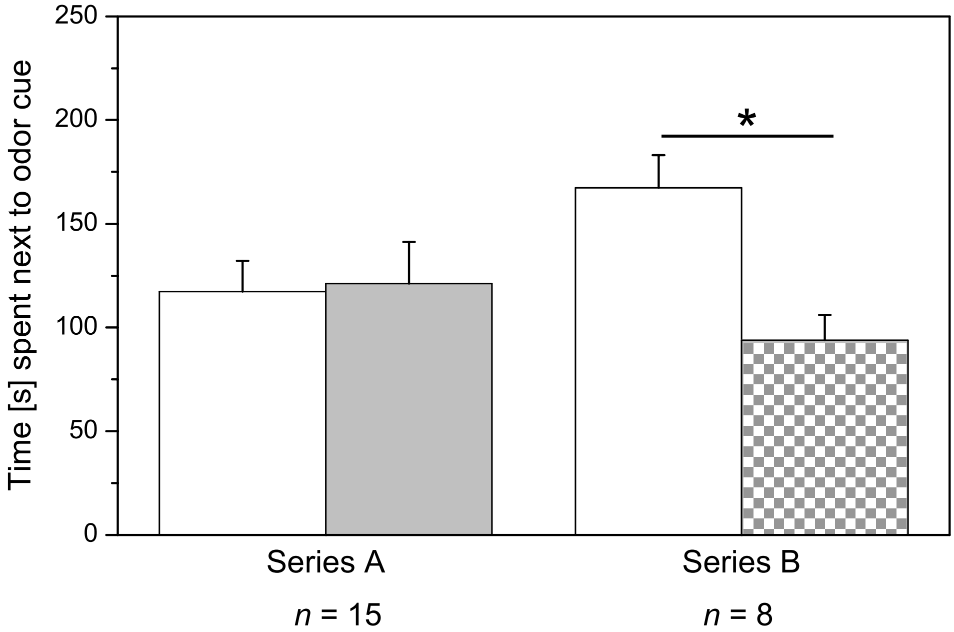

Fig. 3. Mean duration ([s] + standard error, SE) estrous female wood mice spent next to male odor cues within an observational period of 10 minutes; in series A the odor cue of a solitary male = SoM (white box) was compared with the cue of a paired male = PM (gray box); in series B the odor cue of a solitary male = SoM (white box) and the cue of a paired male = PM plus his female partner (squared box) were given. Whereas in series A no significant preference was found, wood mice females significantly avoided the odor cues of the couple in series B.

Discussion

Female wood mice did not prefer heavier over lighter males although they showed a slight tendency to like the heavier of two males better with increasing weight difference between them. Dominance of one male over the other had no influence on female choice. Females did not differentiate between odor of a solitary male and a male already paired with another female; therefore, they did not copy pairing behavior of another female. But females did avoid the combined odor of a pair. Finally and in contrast to previous studies on other small mammals, we could not show any relationship between body weight, social dominance, and urination patterns in male wood mice.

We offer two explanations for the lack of female preference for heavier and/or dominant males. First, female wood mice are not able to differentiate between different males based on odor cues. We do not think that this explanation is likely given the olfactory discrimination capabilities of so many related and unrelated species and their own ability to differentiate kinship odors (Chapter 1). Second, their social structure leads female wood mice to mate rather indiscriminately with the available males to prevent infanticide. We are currently investigating this latter hypothesis. The detailed reasoning follows below.

This lack of female preference for heavier and/or dominant males is surprising because in other small mammals females are able to discriminate between males according to their social status and they prefer dominant males as mating partners. Apps et al. (Apps et al., 1988) and Novotny et al. (Novotny et al., 1990) showed that the quality of urinary odors of subordinate male house mice was altered, reflecting hormonal changes resulting from long-term social subordination. The difference in urine quality from dominant and subordinate males could be detected by female house mice (Hurst, 1990; Rich & Hurst, 1998), bank voles (*Clethrionomys glareolus*) (Hoffmeyer, 1982; Horne & Ylönen, 1996), female hamsters (*Mesocricetus auratus*) (White, 1986) and female brown lemmings (*Lemmus trimucronatus*) (Huck et al., 1981; Huck & Banks, 1982). Female preference for dominant males as mates based on odor contact alone and in real matings has been found in the prairie vole (*Microtus ochrogaster*) (Shapiro & Dewsbury, 1986) and in meadow voles (*M. pennsylvanicus*) (Berteaux et al., 1999). However, females of the promiscuous montane vole (*Microtus montanus*) show no preferences with regard to male dominance (Shapiro & Dewsbury, 1986). Agrell (Agrell, 1997) found that female field voles (*M. agrestis*) preferred dominant males when density was low but showed no preference when male density was high, suggesting that female preference may be context rather than species dependent.

Our own studies have shown remarkable olfactory capability in female wood mice. Estrous females differentiate between odors of unrelated and related males (preferring the former) even without previous contact with the related male (Chapter 1). In the current study females avoided the odor of a pair perhaps so that they might use odors to avoid nest sites potentially defended by other females as shown in house mice (Hurst & Nevison, 1994). Clearly, female wood mice can and do use at least some odor cues to differentiate between males including the subtle differences involved in determining relatedness. This makes the first explanation less plausible.

The alternative explanation is that, in contrast to other species of voles and mice, preference for heavier or dominant males is absent in female wood mice. This could also explain why male wood mice did not express differences in scent marking behavior according to their body weight or dominance rank known from male house mice (*Mus musculus*). The latter exhibit a clear rank-dependent urination pattern with increased marking by dominant males and decreased marking by subordinates (Desjardins et al., 1973). We suggest that these differences might be explained by different mating systems. House mice live in family groups dominated by a single male who sires most of the offspring. Even the odor of a foreign male can cause fetal resorption or abortion (Bruce, 1960). Female wood mice are solitary and mate with several males within one estrus period both in the field and in enclosures (Baker et al., 1999; Bartmann & Gerlach, 2001). Faced with multiple males she may not be able to afford herself the luxury of selecting one partner when males who were not mating partners are infanticidal (Chapter 4). Thus, avoidance of infanticide might outweigh the benefits of mating with heavier or dominant males exclusively. Females do select against closely related males (Chapter 1). It is not known if such closely related males are less infanticidal, a notion that would be suggested by consideration of inclusive fitness.

The relationship between relatedness and infanticide is an interesting remaining question. It also remains to be analyzed if females discriminate against clearly inferior males, e.g. recognizing strains of pathogens and parasites. Recognizing these attributes might be under stronger selection pressure than preferring males of higher body weight or dominance. In this case females are assumed to mate with males with complementary alleles at crucial immune-coding loci.

The results of our odor choice tests now indicate that our earlier results that heavier males had a higher reproductive success than lighter males is not based on female odor preference for heavier males. In promiscuous mating systems the higher reproductive success of heavier males might be caused by factors different from or in addition to female choice. Heavier males could have more or better sperm. They could also have access to females for a longer period or at the most propitious time (Schwagmeyer & Parker, 1987) as shown in rats (McClintock et al., 1982) and bats (Hosken, 1998) where timing of insemination is important in determining fertilization success. In meadow voles (*Microtus pennsylvanicus*), where females also showed no preference for heavier males, however male body mass had a significant influence on the number of copulations performed by males (Berteaux et al., 1999) because females did not distribute copulations evenly among males, and the degree of female preference was related to the difference between the body mass between males.

Acknowledgements We wish to express our gratitude to Jelle Atema for valuable criticism of the manuscript and W. Nagl for statistical advice. We also thank R. Hellmann for laboratory help and H. Markl for generously supporting the project. This study was supported by the University of Konstanz, 'Verband der Chemischen Industrie' and by the Deutsche Forschungsgemeinschaft DFG (Ge 842/1-3). All experiments comply with the currents laws of Germany.

CHAPTER 3: MHC AND MATE CHOICE IN FEMALE WOOD MICE (*APODEMUS SYLVATICUS*)

Keywords *Apodemus* · MHC · Female mate choice · SSCP

Abstract

The major histocompatibility complex (MHC) has been theorized to play a critical role in mate choice selection. Individual discrimination is facilitated by distinctive odors produced by MHC proteins. Variation at two MHC class II genes was examined in wood mice (*Apodemus sylvaticus*). The objectives of the study were (1) to investigate the levels of polymorphism in the MHC complex of this rodent species; and (2) to investigate the influence of MHC genotype in female mate choice decisions, assuming disassortative mating. The amplified gene segments had a low variability (3–6 alleles) compared with other mammalian species. Females did not prefer male genotypes dissimilar to their own. Also no evidence was found for a preference for males with more alleles per locus.

Introduction

The major histocompatibility complex (MHC) is a cluster of approximately 200 genes (Penn & Potts, 1999), divided into three classes of genes that code for cell-surface proteins. The function of these proteins is to bind peptides from invading pathogens and present them to cells of the immune system, thus triggering various immune responses. The genes that make up the MHC are highly polymorphic, with individuals carrying different combinations of these genes. This genetic variation alters the peptide binding site of the encoded proteins, enabling them to bind different foreign peptides.

Numerous studies have shown that MHC genotype is associated with the production of distinctive odors used in the individual discrimination by mice, rats and humans (reviewed by Brown & Eklund, 1994). The mechanism by which MHC type influences urine odor is still unclear, and other genetic differences also influence the ability of mice to discriminate odors (Singer et al., 1997; Singh, 2001).

MHC has been shown to be a marker of relatedness for kin recognition in house mice (Manning et al., 1992), where females prefer communal nesting partners that share allelic forms of major histocompatibility complex genes. In addition, female mice preferentially retrieve pups with the same MHC as their own offspring, and pups are attracted to adult females and other pups with similar MHC odor (Yamazaki et al., 1996). Juvenile Arctic charr (*Salvelinus alpinus*) grow faster when reared with their siblings and these fish can discriminate the odors of their siblings from other individuals even when they have been reared separately since fertilization (Olsén et al., 1998). Recent work indicates that Arctic charr can discriminate the odors of MHC-similar and dissimilar siblings, and they prefer the odor of MHC-similar siblings (Olsén et al., 1998).

Mate choice studies are often only carried out with odor as stimulus for the choosing individual. While a specific odor preference of the focal individual seems to be a good indicator for its real mating preferences, it is important to clarify the role of MHC genotype in mate choice decision. This question has been addressed mainly by work in mice. Yamazaki et al. (1976; 1978; 1988) and Beauchamp et al. (1988) tested mate preference. All but one of six inbred strains of male mice demonstrated a mating preference based on disassortative MHC genotype, that is, a MHC genotype different from the test individual. Egid and Brown (1989) presented evidence that female house mice (*Mus domesticus*) also preferentially mate with males whose MHC haplotype is different from their own. Using mice derived from wild populations in a semi-natural situation, Potts et al. (1991) found consistent deficiencies in the frequency of MHC homozygous progeny compared to expectation under random mating in a series of study populations. After controlling for MHC-related selective fertilization (Wedekind et al., 1996; Rülicke et al., 1998) and abortion (Grob et al., 1998), and having discounted differential mortality in neonates, Potts et al. (1991) concluded that most of the observed homozygote deficit was a product of MHC-disassortative mating. In contrast, Eklund (1997b; 1999) found that wild house mice (*Mus domesticus*), partially cross-fostered in inbred strains, demonstrated no preference for MHC-disassortative partners. Individual pups were exchanged between litters of different strains and were raised in the foster family. The fostering had, in contrast to Penn and Potts (1998) findings, little or no effect on MHC-based mate preferences of wild house mice. Also wild mice did not appear to be using the MHC to avoid inbreeding (Eklund, 1999). While wild female mice may still choose mates based on MHC haplotypes, they do not necessarily prefer MHC-dissimilar mates; additional cues might also be used. These cues might include the male’s dominance rank as MHC-dependent mating preferences have only been found in the laboratory when male dominance has been controlled (Egid & Brown, 1989).

Experimental work on MHC similarity between individuals and odor preferences (Wedekind et al., 1995; Wedekind & Füri, 1997) has suggested that humans can use the MHC in mate choice decisions (but see Hedrick & Löschcke, 1996). Recently a study has been published in which it has been possible to control for balancing selection or patterns of nonrandom mating based on factors other than MHC genotype and show a significant association between genotype at the MHC and mate choice in a human population (Ober et al., 1997). Spouses in the Hutterite population share MHC haplotypes less often than expected. As in mice, human mating preferences appear to be MHC-disassortative, with no general preference or avoidance of specific MHC haplotypes (Wedekind et al., 1995; Ober et al., 1997). As the first clear example of a direct association between MHC genotype and mate choice outside the genus *Mus*, the result of Ober et al. (1997) in humans suggests that the use of the MHC in mate choice decisions may extend across mammals and perhaps all vertebrates.

To mate disassortatively for MHC genes, individuals must have a referent, either themselves (self-inspection) or close kin (familial imprinting), with which to compare the MHC identity of potential mates. Penn & Potts (1998) found that after cross-fostering in house mice (*Mus musculus*) females avoided mating with males carrying MHC genes of their foster family, supporting the familial imprinting hypothesis. Our own work (Chapter 1) on female mate choice in wood mice (*Apodemus sylvaticus*) supports phenotype matching as well, because females in estrus avoid unfamiliar brothers as mates. In this study, we were not able to distinguish between the two mechanisms self-inspection and familial imprinting.

There are three adaptive hypotheses for MHC-dependent mating preferences. First, MHC-disassortative mating preference produce MHC-heterozygous offspring that may have enhanced immunocompetence. Although this hypothesis is not supported by tests against single parasites, MHC heterozygotes may be resistant to multiple parasites (Apanius et al., 1997). Second, MHC-dependent mating preferences enable hosts to provide a “moving target” against rapidly evolving parasites that escape immune recognition (the Red Queen hypothesis). Such parasites are suspected to drive MHC diversity through rare-allele advantage (reviewed in Penn & Potts, 1999). Thus, the two forms of parasite-mediated selection thought to drive

MHC diversity, heterozygote and rare-allele advantage, will also favor MHC-dependent mating preferences. Finally, because MHC genes are highly polymorphic, individuals sharing MHC alleles are likely to be related. Therefore MHC-dependent mating preference may function to avoid kin matings and deleterious consequences of inbreeding (Potts & Wakeland, 1993). This hypothesis is consistent with other evidence that MHC genes play a role in kin recognition.

Furthermore, parental sharing of certain MHC alleles correlates with frequent spontaneous abortions or prolonged intergestational intervals in mice, humans and pigtailed macaques (Potts et al., 1991; Warner et al., 1991; Potts et al., 1994; Jin et al., 1995; Boer, 1995; Knapp et al., 1996). Exposing pregnant female mice to urine of a strange male possessing MHC alleles different from those of the stud led to an increase in resorption of the fetus compared with a confrontation involving two MHC-identical males (Yamazaki et al., 1986).

Our recent work on mate choice criteria in female wood mice (*Apodemus sylvaticus*) demonstrated that female wood mice do not use male body weight, dominance rank, or male urination pattern as a quality sign in choosing their mates.

Drickamer (2000) tested free female choice in house mice (*Mus musculus*) for males independent of investigator-measured or manipulated phenotypic traits. The offspring of pairs with a preferred male as mating partner had a significant higher viability than offspring of non-preferred males. Non-preferred pairs also produced significantly fewer litters. Obviously there are some criteria in female choice, which are not clearly visible, but they nevertheless enhance the females' reproductive success.

In this study we investigated if female wood mice choose their mates with respect to MHC type. Because females are able to identify unfamiliar brothers as mates but did not choose males according to weight, dominance rank or urination pattern in odor tests, we predict a possible underlying MHC-dependent mate choice. We analyzed two MHC genes using the DNA screening technique single-strand conformation polymorphism (SSCP). SSCP is one of the most sensitive methods for rapidly detecting nucleotide substitutions (Hayashi, 1992; Fan et al., 1993; Honey et al., 1993; Law et al., 1996). SSCP analysis relies on the fact that the mobility of a single-stranded DNA molecule in a non-denaturing gel is not only determined by its size, but also by its nucleotide sequence, which governs its three dimensional structure (Rita et al., 1989). Single base pair changes should be detected 99% of the time for 100–300 bp fragments (Less & Apple Baum, 1993) and homozygous and heterozygous animals can be distinguished. We tested the hypothesis if female wood mice prefer disassortative matings using the SSCP data.

Methods

Experimental animals were the F1–F2 generation of wild caught wood mice from different locations in Germany (Konstanz, Reutlingen and Jever); they were assumed to be unrelated. Mice were kept and bred as described in Chapter 1 and 2 of this thesis. Odor preferences of individual estrous female wood mice were tested in a Plexiglas Y-maze as described in Chapter 1 and 2. As odor sources, we used bedding material containing urine cues from single males as described in Kavaliers et al. (1994). After a female mouse was placed in the start box for 5 min, the barrier was removed, allowing the mouse access to the two arms of the Y-maze. During the subsequent 10 min, the behavior of the mouse was videotaped and the time spent in each upstream arm was recorded. ”Preference” was defined as the amount of the time a female spent in one stimulus arm investigating the odor source divided by the amount of time spent actively in the other stimulus arm. Individual trials were discarded if a female spent less than 30 s of the 10 min test in the distal end of the stimulus arms (Kavaliers et al., 1997).

At the end of the experiment, a small piece of tissue was collected from the ear of each individual. The forceps used to collect tissue samples were cleaned after each use to avoid contamination. Pieces of tissue were fixed in 100% ethanol and preserved at 4°C until DNA extraction. Genomic DNA was extracted from each ear tissue sample with the conventional proteinase K/chloroform method (Sambrook et al., 1989). Polymerase chain reaction (PCR) amplification of MHC class II genes was conducted with the primer sets SiSo1&2 (Sommer & Tichy, 1999) and RT1.Ba 390C/RT1.Ba 619NC (Seddon & Baverstock, 1998). SiSo1&2 amplify a variable segment of intron 2 and exon 3 of DQA1 and RT1.Ba 390C/RT1.Ba 619NC amplify the variable second exon of RT1.Ba, a DQA homologue. Exon 2 codes for the first extracellular domain (α1) and the antigen recognition site (ARS; Brown et al., 1988), exon 3 codes for the second extracellular domain (α2). Residues postulated to be involved in the recognition and binding of the antigens, located in the second exon, show high levels of polymorphism (Günther, 1996; Hughes & Yeager, 1998).

The 25 μl reactions contained 1 μl of extracted genomic DNA (10–100 ng), 5 mM KCl, 1 mM Tris-HCl, 0.11 mM $MgCl_2$ (10 x buffer: Amersham Pharmacia Biotech), 0.2 mM dNTPs, 0.5 μM of each primer and 0.625 units of *Red-taq* polymerase (Amersham Pharmacia Biotech). The cycling conditions consisted of 35 rounds of 60s of denaturation at 92°C, 60s annealing at 54°C, and 60s extension at 72°C. A final 5min extension at 72°C followed the last cycle. PCR was performed on a DNA thermal cycler (Biozym). To verify successful amplification, 5 μl of the PCR product was visualized in ethidium bromide-stained 1.5% agarose gels.

2–3 µl of PCR product were mixed with 5–10 µl of denaturating loading dye (prepared after the ETC manual; ETC Elektophoresetechnik), denaturated for 6 min at 95°C, and immediately chilled on ice before loading 7µl of the mixture on the SSCP gel. Polyacrylamide gels (15%; CleanGel/36 samples, ETC Elektrophoresetechnik) were prepared following the manufacturer's protocol and run on a horizontal-cooling electrophoresis system (Pharmacia Biotech). The temperature, power and acrylamide concentration affected the running time and were optimized in a series of trial runs. Maximum separation was reached at constant conditions: 200V, 10mA, 10W for 25 min followed by 400V, 30mA, 20W for 4.5h at 15°C.

After separation, the gels were fixed in 10% acetic acid for 40 min, washed with dH_2O (3 x 10 min) and silver-stained using the PlusOne method (Pharmacia Biotech). Genetic differences between alleles were verified by sequencing homozygous SSCP types from re-amplified double-stranded products.

A test population of 36 wild caught animals from eight different locations in Germany and Switzerland were genotyped to measure the general variability of the MHC loci in wild populations.

The preference of a female indicated by the odor choice test was analyzed with respect to dissimilar or similar MHC genotype of the preferred male. MHC genotypes have been determined for 75 individuals (42 females, 33 males). Some males have been tested twice. Furthermore, a preference for heterozygous versus homozygous males has been tested.

MHC-reflecting female mate choice decisions in a large outdoor enclosure (see Chapter 5) were also analyzed, including a total of 24 litters. The fathers of these litters were assigned as the chosen mates whereas the non-fathers, males resident in the enclosure at the same time as the fathers, were assumed as the non-chosen mates. Altogether 50 individuals (22 females, 28 males) have been genotyped.

Linkage disequilibrium was tested based on unbiased estimates of the exact probability values for the observed combination of alleles calculated with GENEPOP (Raymond & Rousset, 1995). Allele numbers, expected and observed heterozygosity values were calculated, deviations from Hardy-Weinberg equilibrium and the frequency of any possible null allele was estimated for each locus using the program CERVUS (Slate et al., 2000). Significant differences in gene frequencies were tested through F_{IS} values calculated with FSTAT version 2.9.3.2. (Goudet, 1995) based on Weir & Cockerham (Weir & Cockerham, 1984).

Results

Variability in both MHC loci was low in the test population with 4 to 5 alleles per locus and observed heterozygosity ranging from 0.25 to 0.37 (Table 1). No linkage disequilibrium was detected between the two loci.

Table 1. Characteristics of MHC loci of a variety of wood mice from different locations

MHC loci	Individuals typed	Number of alleles	H_O	H_E	HW	Null allele frequency
DQA SiSo1&2*	36	5	0.25	0.70	**	0.47
RT1.Ba§	35	4	0.37	0.64	NS	0.26

* Sommer and Tichy (1999)
§ Seddon and Baverstock (1998)

H_O=observed heterozygosity; H_E=expected heterozygosity
HW=deviation from Hardy-Weinberg equilibrium
**=high significant; NS=not significant

Data of the test population, odor test group (data not shown) and the enclosure population produced similar numbers of alleles per locus and allele frequencies (Table 1, 2).

Microsatellite analyses of 8 different loci were available for the wood mice of the enclosure (Chapter 6). We compared these data with the two MHC loci. The number of alleles was in general much higher for the microsatellite loci (10–14) than for the MHC loci (3–6) and, in contrast to a deficiency of heterozygotes found in the MHC loci, the microsatellite analyses revealed a surplus of heterozygotes (Table 2).

The estimated frequencies of null alleles for all microsatellite loci were slightly negative or close to zero, indicating the absence of null alleles (Summers & Amos, 1997). Estimated frequencies of null alleles for the MHC loci were largely positive, indicating an excess of homozygotes, which could be caused by the presence of a null allele but also by selection against heterozygous individuals and genetic subdivision of the population. Null alleles are a common cause of apparent deviations from Hardy-Weinberg equilibrium, particularly where only a single locus shows a deviation.

Table 2. Characteristics of MHC and microsatellite loci of wood mice from the enclosure

DNA loci	Individuals typed	Number of alleles	H_O	H_E	HW	Null allele frequency
DQA SiSo1&2*	47	6	0.40	0.75	**	0.29
RT1.Ba§	49	3	0.10	0.17	NA	0.24
MSAf-3°	50	13	0.88	0.88	NS	-0.01
MSAf-8°	50	12	0.80	0.80	NS	-0.01
MSAf-16°	50	10	0.94	0.87	NS	-0.04
As-7#	50	11	0.80	0.81	NS	-0.01
As-11#	50	14	0.94	0.89	NS	-0.03
As-12#	50	13	0.80	0.85	NS	0.03
As-20#	50	11	0.82	0.81	NS	-0.01
As-34#	50	12	0.86	0.80	NS	-0.04

* Sommer and Tichy (1999)
§ Seddon and Baverstock (1998)
° Gockel *et al.* (1997)
Harr *et al.* (2000)

H_O=observed heterozygosity; H_E=expected heterozygosity;
HW=deviation from Hardy-Weinberg equilibrium ;
**=high significant ; NS=not significant; NA=not available

Substructuring of populations can occur when family units are localized with low genetic exchange among these units. F_{IS} values were calculated to provide further information on population substructuring (Table 3). Values of F_{IS} are negative when more heterozygotes than expected are present in all groups and positive when more homozygotes are present (Hartl & Clark, 1997).

Table 3. Population genetic structure evaluated by *F* statistic (according to Weir & Cockerham 1984) with FSTAT version 2.9.3.2. (Goudet 1995)

DNA loci	F_{IS}	P
All (MHC + Microsatellites)	0.040	0.030
MHC	0.439	0.025
Microsatellites	-0.018	0.881

While F_{IS} values for the combination of MHC and microsatellite loci and for the MHC loci alone differ significantly from 0, F_{IS} values calculated for the microsatellite loci alone are not significantly greater than 0, indicating that the MHC loci are the driving force for this result. High F_{IS} values indicate an increase in inbreeding in the subpopulation and correspond with the significant deviation from Hardy-Weinberg equilibrium found at these loci.

Corresponding to MHC genotype analyses, males were classified as either “similar” and “dissimilar” or “homozygote” and “heterozygote” in the odor preference tests. Similar males shared all MHC class-IIA alleles with the female whereas dissimilar males expressed at least one different allele than the female. If both males were similar to the female or differ equally from her, these tests were not taken into account. Males were classified as homozygous if only a single allele per locus was detected at both loci and heterozygous if they were heterozygous at least one MHC locus. Tests where males were either both homozygous or heterozygous were not included in the analysis. Females did not prefer the odor of dissimilar males to that of similar males (Mean time [s] ± SE with dissimilar = 103.6 ± 24.8; mean time with similar = 119.8 ± 25.9; Wilcoxon signed-rank test: $n = 22$, $T = 0.480$, $P = 0.402$) (Fig. 1a). They also showed no preference for heterozygote males versus homozygote males (Mean time [s] ± SE with heterozygote = 78.0 ± 20.8; mean time with homozygote = 137.6 ± 54.6; Wilcoxon signed-rank test: $n = 9$, $T = 0.379$, $P = 0.652$) (Fig. 1b).

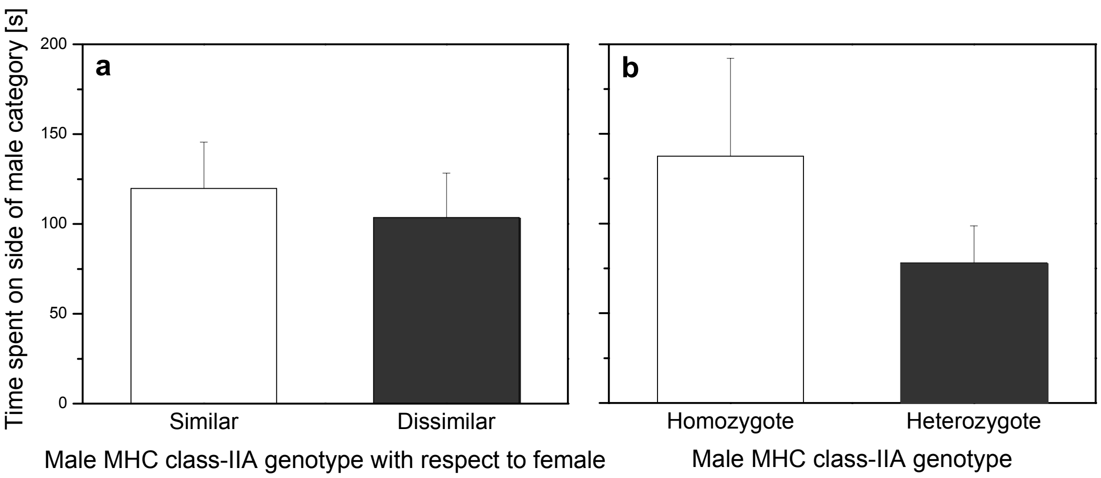

Fig. 1. Odor preference of estrous female wood mice for males in a Y-maze test. **a**, Mean duration ([s]+ standard error, SE) spent on side of male category with either similar (identical to female) or dissimilar (different from female) MHC class-IIA alleles; sample size *n* = 22. **b**, Mean duration ([s] + SE) spent on side of male category either heterozygote (different alleles per locus) or homozygote (one allele per locus) MHC class-IIA genotype; sample size *n* = 9.

Female wood mice mate promiscuously within a single estrus period. As a result, many litters in our enclosure were sired by multiple males. The average number of alleles at the two MHC loci was calculated for the fathers who contributed the litters and the surrounding unmated males. Related males were excluded from the analysis. On average, wood mice female mated with males carrying 2.55 ± 0.42 alleles per two loci, whereas the MHC genotype of the non-fathers had a mean of 2.53 ± 0.33 alleles per two loci. No difference was found in the number of alleles between the two male categories (paired *t*-test: *t* = -0.144, *df* = 32, *P* = 0.886).

Discussion

The test population, consisting of individuals from eight different locations, had relatively low levels of variability at the two MHC loci investigated (4–5 alleles at each MHC locus, SiSo1&2 and RT1.Ba). While microsatellite loci were highly variable in our enclosure population (10–14 alleles per locus), MHC loci had a similarly low variability as the test population (3–6 alleles per locus). Furthermore, the locus SiSo1&2 deviated significantly from Hardy-Weinberg equilibrium. Possible causes of deviations from Hardy-Weinberg equilibrium are population substructure (e.g. family groups; hybridization between subspecies), selection acting on linked loci, biases towards typing particular genotypes, a null allele segregating in the population or a sex-linked locus (Weir, 1996). Population substructure is likely to lead to deviations from Hardy-Weinberg equilibrium at all loci, whereas other causes of deviation from Hardy-Weinberg equilibrium are mostly locus-specific.

Although the population in the enclosure might be sub-structured due to relatedness between selected individuals for this specific analysis, the microsatellite loci of the same individuals showed a completely different pattern from that of the MHC loci. All of the 8 microsatellite loci were in Hardy-Weinberg equilibrium. Therefore, the observed results at the MHC loci are locus-specific. The deficiency of heterozygotes at the MHC loci could be explained by the presence of null alleles, any allele that cannot be detected by the assay used to genotype individuals at a particular locus. A null allele most often occurs due to mutations in one or both primer binding sites, sufficient to prevent effective amplification of the allele. The occurrence of null alleles generally also results in a high proportion of individuals that completely fail to amplify due to the presence of individuals homozygous for the null allele. In this study, no obvious excess of not amplifying individuals was detected. Another possibility to explain the observed pattern might be the incapacity of the SSCP system to detect all alleles. This technique is very sensitive, detecting 99% of single base pair changes (Lessa & Applebaum, 1993). However, 1% of undetected changes remain. Therefore the possibility exists that a single allele determined by SSCP running pattern actually consists of two different alleles, which differ not in their behavior on the gel, but still differ in sequence.

MHC loci in general have been found to be under balancing selection (Hedrick & Black, 1997; Meagher & Potts, 1997; Takahata et al., 1992; Seddon & Baverstock, 1999). Balancing selection, owing to factors such as heterozygote advantage, frequency-dependent selection, or variable selection, maintains variant sequences in a population (Futuyma, 1997). Interestingly, in our study we find an opposite effect at the investigated MHC loci. A possible explanation is furnished by Potts et al. (Potts et al., 1994). MHC loci not only have a function in disease resistance but also in inbreeding avoidance. Wild-derived house mice, experimentally manipulated in levels of inbreeding and MHC homozygosity and heterozygosity, suffered a fitness decline associated with inbreeding but not with MHC homozygosity (Potts et al., 1994).

These data suggest that inbreeding avoidance may be the most important function of MHC-based mating preferences and MHC homozygosity is not necessarily harmful.

The differences between MHC and microsatellite loci are confirmed by F_{IS} values. An F_{IS} value of zero indicates a population with panmictic mating; a negative value is caused by a higher number of heterozygotes than expected and a positive value suggests population sub-structuring (Wahlund effect). As above, a difference between MHC and microsatellite loci has been found. Only the MHC loci alone or MHC loci combined with microsatellites reached significantly positive F_{IS} values, also indicating that the found effect is MHC locus specific.

Sommer (Sommer & Tichy, 1999) investigated two populations of a Malagasy rodent (*Hypogeomys antimena*). While allelic variability of SiSo1&2 was also low in these populations (2 alleles), both populations were in Hardy-Weinberg equilibrium. There was at this locus no variation in two southern elephant seal populations (*Mirounga leonina*), but high levels of variability were detected at this locus in horse (*Equus caballus*) (Slade et al., 1993). A high diversity was found at the other MHC loci RT1.Ba in Australian bush rat (*Rattus fuscipes greyii*) populations (Seddon & Baverstock, 1999), but the variation was substantially reduced on several island populations. Therefore, the variability of MHC loci depends on the species and the condition of the population.

Low MHC variability has been explained (1) by low incidence and transmission of pathogens (Slade, 1992), (2) reduced geographical range and small and declining population sizes (Ellegren et al., 1996) or (3) reproductive strategy and limited dispersal (McGuire et al., 1985; Ellegren et al., 1993; Ellegren et al., 1996). In wood mice, hypothesis (1) seems unlikely because ectoparasites were found on all captured animals (Musolf, personal observation). Hypotheses (2) and (3) were investigated by looking at geographic differences in allele frequencies. No geographic differences were found between allelic distributions in the test population of wood mice (Table 4).

Table 4. Allelic distribution of each MHC locus in the test population

Location	# of individuals	SiSo1&2 alleles	RT1.Ba alleles
Schwanden (CH)	3	a	A, D
Disentis (CH)	3	a	A
Pfäfers (CH)	2	c,d	B
Lengwil (CH)	7	a, b, c, d, f	A,B
Engen (D)	6	a, c, d	A, B, D
Konstanz (D)	5	a, c, d, f	A, B, C, D
Mainauwald (D)	8	a, b, c	A, B
Jever (D)	2	a, b	A, B

Allele “a” of SiSo1&2 is the most common allele all over the different populations, the same pattern is found for allele “A” and “B” of the RT1.Ba locus. The abundance of different alleles seems more likely to correlate with the number of individuals per site than representing a location specific pattern. For a better resolution of allelic distribution more individuals per sites should be investigated.

All animals derived from non-isolated populations. Dispersal is in general not limited in this species. In the enclosure dispersal is partially restricted by fences but because of the occurrence of immigration (Chapter 6), we assume that emigration also was possible. The promiscuous mating system in general should lead to a higher variability in alleles. Therefore we reject the hypotheses (2) and (3). In summary, causes for the heterozygote deficiency at the two MHC loci are unclear.

The results of the two mate choice experiments are surprising. Females did not show a preference for the odor of a dissimilar or of a similar male. Heterozygosity of the male also had no influence on female decisions. Furthermore, in the enclosure, females chose their mates regardless of the number of alleles a male had.

House mice (*Mus domesticus*) and humans mate disassortatively with respect to MHC loci and demonstrate no general preference or avoidance of specific MHC haplotypes (Egid & Brown, 1989; Potts et al., 1991; Wedekind et al., 1995; Ober et al., 1997). In mice, this preference can be influenced by cross-fostering and the dominance rank of males (Egid & Brown, 1989; Eklund, 1999). The results of our study may be explained by MHC-independent mate choice. Female wood mice are indiscriminate in choosing mates of different quality (Chapter 2), including the dominance rank of the male. Instead, they mate multiple times with all males in the neighborhood confusing paternity and protecting their litters from infanticidal males (Chapter 4). This effect obviously has stronger impact on female mating behavior than variation in male traits, possibly including different MHC genotypes. Furthermore, the lack of preference for MHC genotypes might also be due to an experimental error in the determination of different MHC alleles caused either by the presence of null alleles or the insufficiency of detecting all alleles with the SSCP method.

Previous studies suggested MHC-disassortative mating could be a possible means of inbreeding avoidance, might be used to increase the MHC heterozygosity (Potts & Slev, 1995; Penn & Potts, 1999). Focusing on the distinction between similar and dissimilar MHC genotypes may not be efficient when parasite resistance is important. In these situations, females should choose partners that maximize the number of different MHC alleles in their offspring (Penn & Potts, 1999). In female sticklebacks (*Gasterosteus aculeatus*), males with many alleles were chosen in mate choice decisions whereas the similar or dissimilar genotype had no influence (Reusch et al., 2001). These results were based on the analysis of 6 different MHC class-IIB loci. The reduced variability and the low number of different loci used in our study on wood mice could contribute to the lack of preference for males with few or many different MHC alleles. Further MHC loci should be analyzed to become better insight in MHC dependent mating patterns.

In addition to MHC proteins, rodent urine also contains a class of proteins, termed major urinary proteins (MUPs) that bind and release small volatile pheromones. In wild house mice (*Mus domesticus*), the combinatorial diversity of expression of MUPs might be as great as for MHC, and at protein concentrations a million times higher, might play a particularly important role in the individual recognition mechanism (Hurst et al., 2001). Therefore, MUPs should be included in mate choice investigation as well.

CHAPTER 4: MATING WITH THE ENEMY: POLYANDRY AS INFANTICIDE AVOIDANCE

Keywords *Apodemus* · Female multiple mating · Infanticide avoidance

Submitted to *Current Biology*

Abstract

Male reproductive success typically increases with the number of mating partners, but for females, offspring production is generally maximized with far fewer matings. Nevertheless, females of most species mate with multiple males (polyandry), although why they do so is unclear. We present for the first time evidence that polyandry reduces male infanticide by keeping them in the dark about offspring paternity.

Methods, Results & Discussion

Polyandry is often assumed to be a strategy to increase fertility (Hoogland, 1998), increasing the genetic variability of offspring (Tooby, 1982), avoiding genetic incompatibility (Zeh & Zeh, 1996) or inbreeding (Hosken & Blanckenhorn, 1999). However, females may also mate multiply to confuse the issue of paternity in order to avoid infanticide by males. For example, it is well known that the risk of violence and death in human children is greatly increased if they live with males who know that they are not their genetic fathers (Daly & Wilson, 1988). However, while infanticide may favor mechanisms that confuse paternity as it has been assumed by Hrdy (1979), there is no experimental support for the idea that multiple mating leads to reduced rates of infanticide. We tested if litters of wood mice (*Apodemus sylvaticus*) sired by multiple males had a higher chance of survival than litters of a single father. Previous experiments in the wild (Baker et al., 1999) and in an outdoor enclosure (Bartmann & Gerlach, 2001) showed that many litters were sired by between 2 and 4 males.

For each experiment we introduced 1 female and 3 male wood mice into 4 cages interconnected by translucent PVC pipes. All animals were sexually inexperienced but older than 8 weeks and hence sexually mature. We counted the number of offspring of each female produced across three litters. When infanticidal behavior occurred all pups of a litter were found dead by bites at the neck or head. The dead pups were removed from the cage. Tissue samples of these animals as well as of all potential fathers, the mothers and weaned offspring were collected. To assign paternity, alleles of all offspring were compared with those of the mother and all potential fathers using DNA microsatellites (Harr et al., 2000). Analysis showed that infanticide happened only in one (11%) out of 9 litters which were sired by multiple males, but in 19 (53%) out of 36 litters which were sired by only one male (Fishers exact test, $chi^2 = 5.06$, $P = 0.031$). Male attacks against pups were occasionally observed and indicated the attackers were indeed males who had not sired the pups. It has previously been shown that infanticidal behavior in males decreases significantly with ejaculation in house mice (Perrigo et al., 1990), and therefore male infanticidal behavior ceases by the time his own offspring are born three weeks later. We suggest a similar mechanism here.

Female wood mice re-mate immediately after giving birth. Killing the litter reduced the gestation duration of females significantly (from 27.2 days to 23.6 days; ANOVA, $F = 34.6$, $P = 0.0001$). Therefore, infanticidal males can potentially mate sooner with the mother of the killed pups than they would have otherwise, and hence increase their reproductive success over their short life span of just a few months. Males are apparently not able to differentiate between their own pups and those sired by other males. To avoid killing their own pups they accepted the whole litter.

Finally, because females losing a litter which was sired by a single male are significantly more likely to re-mate with multiple males than if their first litter survived (Fisher exact test, $chi^2 = 6.2$, $P = 0.028$), polyandry appears to be an active female strategy to avoid infanticide.

We anticipate that avoidance of infanticide will help explain the evolution of female promiscuous matings in many species. For example, it has been suggested human females developed hidden estrous so they can confuse paternity, and as a mechanism to facilitate monogamous pairbonds inducing males to stay close to the female at all times since males can never know when ovulation will occur. It may also serve as a means of protecting females and offspring from the attacks of cuckolded males. Whatever the case, avoidance of infanticide is an important driving force in the evolution of many mating systems and should be considered in mating pattern investigations.

CHAPTER 5: CHARACTERIZATION AND ISOLATION OF DNA MICROSATELLITE PRIMERS IN WOOD MICE (*APODEMUS SYLVATICUS*, RODENTIA)

Keywords Microsatellite loci · *Apodemus sylvaticus* · *Apodemus flavicollis* · Rodentia

Published in *Molecular Ecology* 9: 1661-1686 (2000)

The wood mouse (*Apodemus sylvaticus*) widely distributed in European forests and bush land is an interesting model animal to study the evolution of social behavior and mate choice. In this context we were interested in the dynamics of the genetic structure of a population over several years that might indicate clustering of kin groups and recruitment of subadults. Mitochondrial DNA studies revealed little variation in this species (Gerlach, unpublished results) and stressed the need for the development of microsatellite markers to accurately assess and monitor genetic variation and changes. In this study we describe the identification of highly variable microsatellite markers, which will provide a powerful tool for the investigation of the genetic structure of wood mice populations. DNA from muscle tissue from an individual of *Apodemus sylvaticus* was extracted as described in Sambrock *et al.* (1989) using the conventional proteinase K/chloroform method. Genomic DNA was digested with the restriction enzyme *SAU*3A I and electrophoresed in a 0.8% agarose gel (Rassmann et al., 1991). DNA fragments were excised and purified from a range of 400 to 900 bp. A size-selected library was constructed by ligating the DNA fragments into the vector pUC 18 (Pharmacia) following the manufacturer's recommendations. Plasmids were transformed into electrocompetent *Escherichia coli* XL-1 Blue cells by electroporation (E. coli pulser, Bio-Rad) and grown on agar plates. Approximately 20000 recombinant plaques were blotted on Hybond N+ nylon membranes (Schleicher and Schüll) and were screened using digoxygenin labelled dinucleotide $(GT)_7$ and $(GA)_7$ using standard hybridization techniques (Schlötterer, 1993). Forty-eight positive clones which showed a signal on the X-ray film after chemiluminescence detection (CSPD) were sequenced using the ABI PRISM dye terminator cycles sequencing-ready reaction kit (following the recommendations of the manufacturer) and analyzed with an ABI PRISM 377 automated sequencer. Eight dinucleotide repeats (Table 1) could be revealed and primer sets were designed with the help of the program OLIGO (National Biosciences Inc., Version 4.0). They amplified repeatably and were polymorphic within a population of 30 individuals. These animals were live-trapped at different localities near Konstanz, Southern Germany. Before releasing the animals pieces of tissue were collected from the ear, fixed with 100% ethanol and preserved until DNA extraction.

Allelic variability and heterozygosity of the loci was determined with DNA extracted from tissue samples of wood mice according to standard protocols (Sambrook et al.,

1989) with proteinase K treatment. PCR amplification (Saiki et al., 1988) was carried out in a DNA thermal cycler (Biozym) in 10 µl of reaction mixture including about 10–100 ng of template DNA, 0.2 mM dNTP, 0.5 µM of each primer, ddH_2O, 1 x PCR buffer (Amersham Pharmacia Biozym, 50 mM KCl, 1.1 mM Mg_2Cl, 10 mM Tris-HCl), and 0.25 units of *Red-taq* polymerase (Amersham Pharmacia Biozym). 25 PCR cycles were performed: denaturation at 94°C for 1 min, annealing for 1 min (temperatures listed in Table 1) elongation at 72 °C for 1 min and a final elongation step for 10 min. 2 µl PCR product were applied to a 60 cm long 6% denaturing polyacrylamide gel, blotted onto positively charged nylon membrane, detected by probing with GA/GT oligonucleotides labeled with the DIG system (Boehringer Mannheim) and visualised by chemiluminescence (CSPD) according to the supplier's protocols (Boehringer Mannheim) (Schlötterer, 1993). The genotypic independence between loci was tested, based on unbiased estimates of the exact probability values for the observed combination of alleles calculated with GENEPOP (Raymond & Rousset, 1995). No linkage disequilibrium was detected. Allele numbers, unbiased expected and observed heterozygosity values were calculated using the program GENETIX (Belkhir et al., 1997) shown in Table 1. Observed heterozygosity ranged from 0.74 to 0.97 and number of alleles per locus was relatively high with 7 to 15 different alleles. Amplification was tested for all primer sets for the second, sympatric living species *Apodemus flavicollis* (Table 2), which is closely related to *A. sylvaticus* (Martin et al., 2000). Only three out of 6 primers developed from *A. sylvaticus* DNA amplified PCR-products in *A. flavicollis*. Although number of alleles found in *A. flavicollis* (11) was similar to *A. sylvaticus* different allelic patterns were observed in *A. flavicollis* and observed heterozygosity (0.58 – 0.71) was slightly less than that of *A. sylvaticus*. These preliminary results indicate the potential utility of microsatellite DNA markers in future studies of populations substructuring and gene flow in wood mice.

Table 1. Microsatellite loci in wood mice, *Apodemus sylvaticus*. Size range refers to the observed PCR product sizes. Number of alleles was determined from 30 individuals. Animals were caught at different places in the wild and therefore were assumed to be unrelated. (N_A: number of alleles, H_O: observed heterozygosity, H_E: unbiased expected heterozygosity, T_A: annealing temperature). The sequences were submitted to Genbank (acc: accession numbers)

Locus acc	Size range	Repeat in clone	N_A	H_O	H_E	T_A (°C)	primer sequence (5'-3')
As–7 AF246520	114	$(GT)_{19}$	10	0.74	0.81	47.8	F: CAGGTCTTATTCTTCCAGTTA R: ACAATTGATTAAATTGGAACC
As–11 AF246521	248	$(GT)_{23}$	15	0.97	0.90	50.5	F: GGAAGTTTAGTGGTCTGGTG R: GATCAGGATTTCCTAGAAAGAA
As–12 AF246522	249	$(TG)_{22}$ $(GA)_{24}$	14	0.73	0.88	53.6	F: TGTCAGGTCTCAACAGTAGG R: CTGTTTGGAGTTGTTGTTCTG
As–20 AF246523	144	$(GT)_{25}$	11	0.84	0.86	55.0	F: CAGGTGAACACCCTCCCATAA R: AGCCACAGAGCCAATAAGAAG
As–27 AF246525	138	$(AG)_{19}$	7	0.85	0.86	55.0	F: TGATTTGACCCTATGAG CAG R: CCCACACCACATGCCATACAC
As–34 AF246526	150	$(AC)_{18}$	12	0.84	0.84	47.1	F: CCAGAAGTATGCTGTGGTTTT R: TTAAGAATGACTAAGGATCAG

Table 2. Microsatellite loci in yellow necked mice (*Apodemus flavicollis*). Cross-species amplification was tested with 24 individuals from different places near Konstanz, southern Germany, – indicates that primers did not amplify PCR products

Locus	N_A	H_O	H_E
As-7	11	0.58	0.87
As-11	–	–	–
As-12	11	0.61	0.88
As-20	11	0.71	0.89
As-27	–	–	–
As-34	–	–	–

Acknowledgements We would like to thank Prof. Hubert Markl for supporting the project and R. Hellmann for laboratory help. This study was supported by the Verband der Chemischen Industrie and the Deutsche Forschungsgemeinschaft DFG Ge 842 (1–1, 3).

CHAPTER 6: SOCIAL STRUCTURE OF A SEMI-WILD WOOD MICE POPULATION (*APODEMUS SYLVATICUS*)

Keywords *Apodemus* · Territory inheritance · Reproductive success

Abstract

The study of a wood mouse (*Apodemus sylvaticus*) population in a 400m^2 enclosure over 32 months confirmed the high frequency of multiple paternity in this species as 82% of litters were sired by 2–3 males. Inbreeding avoidance and/or avoidance of infanticide of males by confusing paternity are discussed as causes for multiple matings by females. Increased body weight significantly increased the reproductive success of males whereas females' reproductive success depended significantly on the time spent in the enclosure. In connection with the stay in the enclosure the quality of nest sites played a major role in female reproduction. Here we demonstrate the first evidence for territory/nest site inheritance in female wood mice under semi-natural conditions. Females who inherited the maternal territory had a higher reproductive success than other females in the enclosure. Although remaining in the mother's territory results in reproductive suppression of the daughters, the possibility of inheriting a high quality territory may exceed the delay to first reproduction, especially when territory availability is low.

Introduction

The social structure of a species can be described by the spatial and genetic relationship between the individuals. Dispersal influences the genetic structure of a population, resulting in variably sized kin clusters within which special social behaviors may evolve. Philopatry will favor the evolution of cooperative traits between members of the sedentary sex (Greenwood, 1980).

In mammals females tend to remain at or near their natal site (Greenwood, 1980). Philopatry should lead to an increase in relatedness between neighboring breeding females if young females become sexually mature near their mothers and sisters. A correlation between geographic distance and relatedness has been shown in meadow voles (*Microtus pennsylvanicus*) (Sheridan & Tamarin, 1986). Matrilines, groups of related females, have been found in Richardson's ground squirrels (*Spermophilus richardsonii*) (van Staaden et al., 1994), yellow-bellied marmots (*Marmota flaviventris*) (Armitage & Schwartz, 2000), and gray-sided voles (*Clethrionomys rufocanus*) (Ishibashi et al., 1997). In some species matrilines are only found at specific times in the year (McShea & Madison, 1984; Kawata, 1990; Pusenius et al., 1998) or at critical population densities (Lambin & Krebs, 1991a). Kinship between females in bank voles (*Clethrionomys glareolus*) (Mappes et al., 1995) and Townsend's voles (*Microtus townsendii*) (Lambin & Krebs, 1993) leads to substantial overlaps in the home ranges without decreasing the reproductive success of these females. Breeding success is higher in kin groups as well (Lambin & Krebs, 1993; Mappes et al., 1995; Solomon & French, 1997).

What advantages does an animal receive if it decides to stay? Dispersing may be dangerous: new territories must be conquered, predation pressure is increased and food availability is insecure. On the other hand, living in a kin cluster has various costs as well. As inbreeding decreases fertility and survival of offspring (Ralls et al., 1986; Charlesworth & Charlesworth, 1987) inbreeding depression is assumed to be a cost of staying in the proximity of relatives. In marmots (*Marmota*) the cost of sociality (offspring staying with their parents after weaning) is reproductive suppression (Armitage, 1999). However, this reproductive loss is partially compensated by increased survivorship and alloparental care and subordinate adult marmots may also reproduce or succeed to territorial status (Armitage, 1999). Often the puzzling behavior of helping is observed (see Emlen, 1991; Solomon & French, 1997; Stacey & Koenig, 1990, for reviews). Here individuals forego opportunities to reproduce and help others to breed. Kin selection with the gain of indirect benefits (Hamilton, 1964) may result in a selective advantage to (related) helpers and direct benefits may also be important. Helpers may benefit by gaining protection and feeding benefits in the established territory (Gaston, 1978; Taborsky, 1984; Taborsky, 1985), by increasing the probability of mate or territory acquisition (Reyer, 1980; Queller et al., 2000), and by increasing the probability of their survival through group augmentation (Taborsky, 1984; Brown, 1987).

The cooperatively breeding cichlid fish *Neolamprologus pulcher* must pay with help in order to remain protected in the family group and may ultimately inherit the natal territory (Balshine-Earn et al., 1998). In Columbian ground squirrels, the daughters inherit maternal nest sites (Harris & Murie, 1984) that may help them become more easily established and reproduce successfully.

Not only territory inheritance but also rank inheritance could be an important criterion in the decision to stay. Many species of Cercopithecines (Primates) exhibit a matrilineal dominance system in which a female inherits her mother's rank (or acquires her genealogical rank) (de Waal, 1977; Walters, 1980; Datta, 1983; Horrocks & Hunte, 1983; Chapais, 1988).

In addition to helping cooperative breeding may also serve as an alternative strategy to dispersal. Communally nesting house mouse females (*Mus musculus domesticus*) suffer lower rates of reproductive suppression (König, 1994) and infanticide (Manning et al., 1995), and exhibit more efficient energy balance in converting food into milk and/or milk into offspring growth (König, 1993) compared to single nesting females. Kin were also preferred as nesting partners in this species (Manning et al., 1992) and kin groups received higher reproductive success compared to groups of unrelated females (König, 1994) suggesting inclusive fitness benefits to communal nursing.

In wood mice (*Apodemus sylvaticus*), breeding females have mutually overlapping homes ranges (Wilson et al., 1992), but kinship between neighboring females has not been demonstrated. No sex specific dispersal tendencies have been found in wood mice (Schulte, 1997). In yellow-necked mice (*Apodemus flavicollis*), a closely related species, a tendency for association of kin in winter and spring has been shown with the analysis of the mtDNA control region (Rosakis, 1999).

Telemetry studies on wood mice have revealed communal use of nest sites of reproductive females under natural conditions (Wilson et al., 1992). In an enclosure experiment, communal nests with litters of two different females were identified (Bartmann & Gerlach, 2001). Gerlach and Bartmann (2002) investigated the reproductive success of breeding units of two wood mouse females with different degrees of relatedness with respect to costs and benefits each member has experienced. In contrast to findings in the closely related house mouse where communal breeding improved lifetime reproductive success (König, 1994), no such effect could be found in wood mice. Rather, at least one or both females had to pay for this cooperation in terms of less weaned own offspring and/or more investment by nursing the offspring of the partner. Based on these results, solitarily breeding should be preferred in wild wood mice. Under harsh environmental conditions such as low nest site availability and food limitation other advantages may support cooperative breeding. Under these circumstances, daughters always could increase their inclusive fitness by helping their mothers to wean offspring.

The mating system is an important factor determining the social structure of a species. Wood mice are considered as promiscuous (Bartmann & Gerlach, 2001); one litter could be sired by multiple males and the siblings could be half-sibs. The degree of relatedness within one litter could therefore be as low as between litters of different females with the same group of fathers. This low degree of relatedness all over the population probably influences kin related behaviors.

The variance in individual reproductive success is also correlated with the mating system. While typical mammalian polygyny is expected to increase the variance in individual reproductive success of males, monogamy should have a reverse effect, ensuring an equal share of reproduction among males and females in a population. Promiscuity in wood mice led to equal individual reproductive success (Bartmann & Gerlach, 2001), although a large male advantage was found which was explained with sperm competition.

The aim of this study is to investigate a wood mice population under semi-natural field conditions in a large enclosure. Genetic analyses of field data have failed to clarify the mating system and the social structure of this species. Our enclosure is large enough to prevent cage artifacts but small enough to enable recording of all animals. We are especially interested in female breeding behavior. We tested the following question: Is there any incidence of social behavior among wood mice females in the wild? If so, what benefits does a female gain by being social?

Methods

We studied a semi-natural wood mice population in an outdoor enclosure from May 1997 until December 1999. The 400 m^2 large enclosure was situated in the forest near the University of Constance. The enclosure was fenced with 1 m metal sheets, which were dug 0.5 m in the ground to limit escapes of captive wood mice. However, immigration of strange individuals occurred in low numbers throughout the whole study.

Food pellets were offered at five sites in the enclosure *ad libitum*. Ten nest boxes each with two connecting tubes were dug 0.5 m in the ground as well. Wood mice visited and used these boxes often but no breeding took place in the artificial nests.

We established a breeding population with 5 male and 5 female wood mice in May 1997 using the F1 generation from various lines of wild caught wood mice breeding in our laboratory. Mice were individually marked by tattooing the tail with a color code (Hugo, 1990). Mice were trapped weekly in live traps (Luna mouse traps, DeuFa Neustadt/Inn, Germany). Body weight, breeding condition and the location of the animals were recorded. Newly appearing animals were sexed, weighted and marked as well. Before release, a small piece of tissue was collected from the ear of each individual. The forceps used to collect tissue samples were carefully cleaned after each use to avoid contamination. Pieces of tissue were fixed in 100% ethanol and preserved at 4°C until DNA extraction.

Genomic DNA was extracted from each ear tissue sample with the conventional proteinase K/chloroform method (Sambrook et al., 1989). Polymerase chain reaction (PCR) amplification and genotyping were conducted as described in part by Harr et al. (Harr et al., 2000) for 8 microsatellite loci: MSAf-3, MSAf-8, MSAf-16, As-7, As-11, As-12, As-20, As-34. The PCR products were separated on polyacrylamide sequencing gels, blotted on to a positively charged nylon membrane, stained with digoxygenin-labeled GA or GT oligonucleotides (Boehringer Mannheim) and visualized by chemiluminescence (CSPD) by means of X-ray-sensitive film according to the supplier's protocols (Boehringer Mannheim). The alleles found at the loci were numbered according to their basepair length.

Allele frequencies were calculated and parentage analyses were performed using CERVUS 2.0 (Marshall et al., 1998). When using the allele frequencies found within our enclosure population, we have a total exclusion power for the combination of all 8 loci for the first parent of 0.996 and for the second parent of 0.999.

We assumed that 80% of candidate males were sampled, 100% of loci were typed, and a 1% rate of typing error. The simulation program within CERVUS 2.0 was used to estimate the critical difference in log-likelihood scores between the most likely and second most likely candidate parent for assignment of parentage at a level of 95% and 80% confidence. The analyses presented here are based on relaxed (80%) statistical confidence, which is reasonably accurate determining mating success (Slate et al., 2000), although 61% of paternity analyses were determined with 95% confidence.

Results

The number of individuals in the enclosure during the 2.7 years ranged from 1 to 20 animals (Fig. 1) with high numbers in summer and autumn and low numbers in winter and spring. Juveniles and subadults were found primarily in late summer to early winter. Immigrants were found in late summer to early autumn or in early spring. No winter reproduction was found. In spring and summer 1999 the number of animals was comparably low (Fig.1). This followed long lasting and heavy rainfalls, which caused the extreme flooding of Lake Constance and also led to very humid conditions in the enclosure.

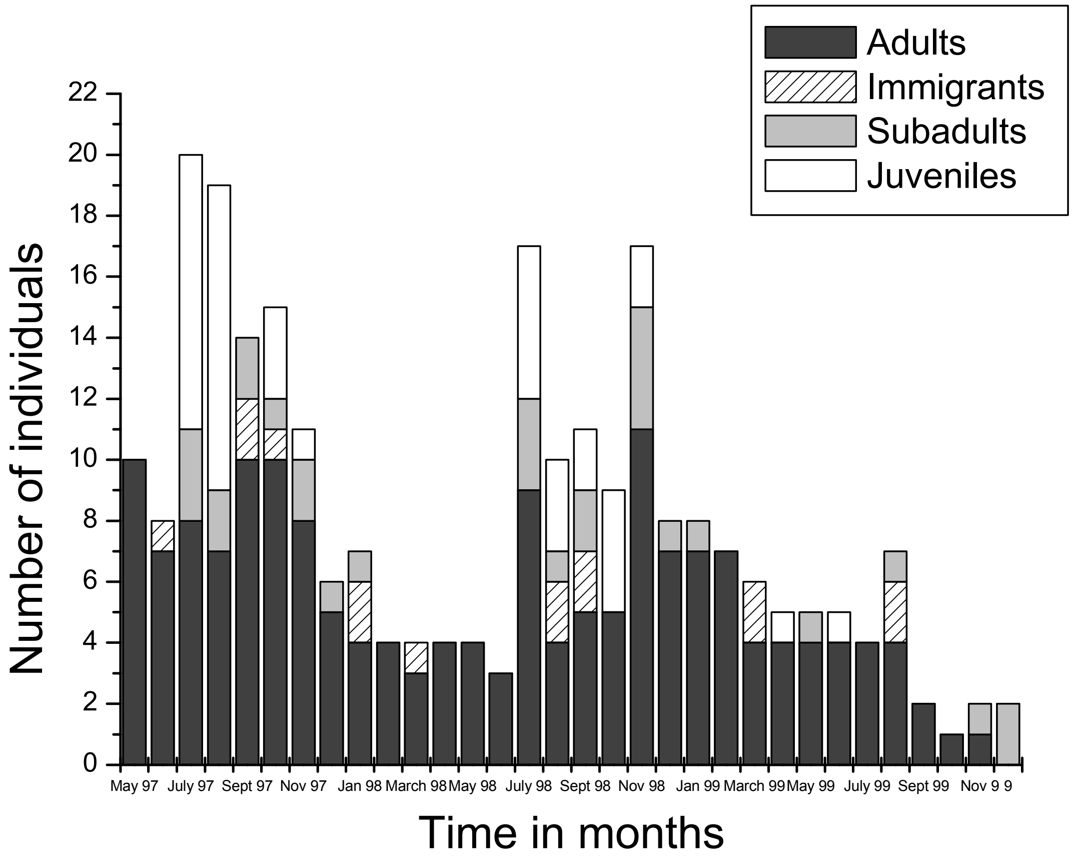

Fig. 1. The number of trapped wood mice per month in the enclosure starting in May 1997 ending in December 1999. The categories adult, subadult and juvenile were determined by weight: juvenile ≤ 14 g, subadult 14 g $< x >18$ g, adult ≥ 18 g. Individuals with no genetic relationship with the enclosure population have been classified as immigrants.

No difference was found between the number of females and males trapped in the enclosure in the seasons spring, summer, autumn and winter (Wilcoxon signed-rank: $df = 11$, $T = 0.464$, $P = 0.484$). When the different age categories (juvenile ≤ 14 g, subadult 14 g $< x > 18$ g, adult ≥ 18 g) were considered individually no difference in presence between the sexes was found (Wilcoxon signed-rank: $df = 11$; $T_{ju\ v}= 0.162$, $P_{juv} = 0.250$; $T_{sub} = 0.600$, $P_{sub} = 0.805$; $T_{adult} = 0.949$, $P_{adult} = 0.965$). Although altogether more males ($n = 71$) than females ($n = 49$ females) were trapped during the study.

The 8 microsatellite loci were characterized using the 122 individual wood mice: 49 females, 71 males and 2 unsexed baby mice (Table 1). No locus contained an excess of homozygotes or deviated from Hardy-Weinberg equilibrium. The overall probability of exclusion for the set of 8 loci used in the parentage analyses was calculated in CERVUS 2.0 using the actual allele frequency data for the 122 mice. The probability of exclusion was 0.996 for the first parent and 0.999 for the second parent (assuming that the first parent was assigned correctly).

Table 1. Characteristics of loci used in paternity analysis of wood mice juveniles conceived in 1997–1999
H_O=observed heterozygosity; H_E=expected heterozygosity

Microsatellite loci	Individuals typed	Number of alleles	H_O	H_E
MSAf-3*	122	13	0.828	0.865
MSAf-8*	122	14	0.836	0.804
MSAf-16*	122	10	0.834	0.854
As-7§	122	12	0.811	0.789
As-11§	122	15	0.893	0.890
As-12§	122	14	0.820	0.837
As-20§	122	11	0.762	0.764
As-34§	122	14	0.828	0.781

* Gockel *et al.* (1997)
§ Harr *et al.* (2000)

During the whole study 16 wood mice (7 males and 9 females) were introduced from the lab in the enclosure. Of the reminding 106 new animals trapped in the enclosure a mother was assigned to 91 (86%) and a father was found for 90 (85%). For 15 animals no mother and father was identified. These animals were considered as unrelated immigrants in the enclosure. Males (n = 13) immigrated significantly more often than females (n = 2) (chi^2 = 4.09; df = 1; P = 0.0431). Only 20% of immigrants became established in the enclosure. The occurrence of immigration per month (n = 32) was not influenced by the actual population size in the enclosure but significantly depended on the season and the population size crossed with the season (ANOVA: F_{Season} = 7.590, df = 11, P_{Season} = 0.004; $F_{Pop\text{-}size}$ = 0.828, df = 1, $P_{Pop\text{-}size}$ = 0.400; $F_{Season*Pop\text{-}size}$ = 6.882, df = 11, $P_{Season*Pop\text{-}size}$ = 0.056). No difference between years was found (chi^2 = 1,2; df = 2; P > 0.5).

The offspring could be organized in 34 litters; mean litter size was 2.8 offspring (range: 1–6). 12 single offspring litters and 22 litters consisted of 2–6 offspring. 82% of litters with more than a single offspring were sired by more than one male (2–3 males). Inbreeding was determined in three cases: a sister-brother mating, a mother-grandchild mating and a mother-nephew mating. In two cases also an unrelated male sired partially the litter.

Individual reproductive success of adult females (n = 18) and males (n = 30) ranged between 0 and 24 offspring produced per female and between 0 and 10 offspring per male. On average, female had 5.1 ± 6.5 offspring and males had 2.5 ± 2.8 offspring. A significant difference in the variance of the reproductive success was found between sexes with females having a higher variance than males (Levene test for unequal variances, $F_{1,46}$ = 16.4, P = 0.0002). The most successful female weaned 26% of all offspring (8 litters with together 24 pups), whereas the most successful male sired 11%. The difference in variance between the sexes remains significant (Levene test for unequal variances, $F_{1,45}$ = 14.1, P = 0.0005) with the removal of the female with the most reproductive success.

A possible relationship between reproductive success of animals, body weight and time they spent in the enclosure was analyzed. The weight was taken at the age of approximately two months, at which stage animals were considered as adults and becoming sexually mature. In this way, introduced animals from the lab can be compared with wild born animals and female pregnancy had no influence on weight yet.

Females had a mean body weight of 21.6 ± 3.5 g and they stayed on average 5.1 ± 4.8 months in the enclosure. Whereas female body weight had no influence on the number of offspring a female produced (ANOVA, df = 1, F = 0.06, P = 0.81), the stay in the enclosure is highly significant and positively correlated with the number of produced offspring (df = 1, F = 35.17, P < 0.0001).

Male wood mice had a mean weight of 23.7 ± 4.5 g and were trapped in the enclosure on average for 3.3 ± 2.6 months. In this sex, while the residence did not influence the produced offspring (ANOVA, df = 1, F = 0.27, P = 0.61) body weight had a significantly positive effect (df = 1, F = 14.50, P = 0.0007).

No significant difference between male and female body weight was found

($chi^2 = 3.63$; $df = 1$; $P = 0.06$).

Matrilineal structures of breeding females were found in the enclosure (Table 2).

Table 2. Wood mouse females' presence in the enclosure from May 1997 to December 1999. Dark gray boxes indicate non-reproductive stages, light gray boxes indicate reproduction. Numbers within boxes stand for offspring produced. With females W1–W5 the experiment started, W2 and W3 were sisters, al well as W4 and W5. W6/W7, W8/W9 were later introduced from the lab, both sister pairs. All other females are the F1–F2 generation of these animals, numbers stand for origin. W10 was an immigrant female, no parents could be found with the microsatellite analysis

	1997								1998												1999											
Female	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
W1	▓	▓																														
W2	▓																															
W3	▓	▓	░	░	░ 10	░	░	▓																								
W3.1									▓																							
W5	▓	▓	░	░ 10																												
W4	▓	▓	░	░	░ 13	░	░	▓																								
W4.1				▓	▓	░ 0	▓																									
W4.2				▓	▓	▓	▓	▓	▓	▓	▓	▓	░	░	░	░ 24	░	░	░	░												
W4.2.1													▓	▓	░	░ 8	░	░	▓	▓	▓											
W4.2.2													▓	▓	░	░ 3																
W4.2.3																▓	▓															
W4.2.4																		▓	▓	▓	▓	▓	▓	░	░	░ 9	░	░				
W4.2.5																			▓	▓	▓	▓	▓	▓	▓	░	░	░ 9	░	░	░	
W6															▓																	
W7															▓																	
W8																			▓	▓	▓	▓	▓	▓	▓	░ 1						
W9																			░ 2													
W10																												░ 2				

We considered females who were adults when released in the enclosure or female offspring who reached adulthood in the enclosure. From the five introduced females W1–W5 in 1997 only three reproduced successfully. Daughters remained in the enclosure and reproduced as well. The most successful female W4.2 (numbers stand for the origin of the female), a daughter of W4, recruited five daughters (W4.2.1–W4.2.5) who either bred together with the mother in 1998 or in the following year when the mother died. Newly introduced animals from the lab W6–W9 had variable success in breeding. Females of the most successful matriline produced on average 8.44 ± 7.40 offspring whereas the females of the other matrilines gave birth to average 1.67 ± 3.24 (ANOVA: *df* = 1; *F* = 6.33, *P* = 0.02).

Only three to four females bred concurrently in the enclosure even when more females were available for breeding (Table 2).

The area of most frequent trapping places of each female was recorded. Whereas some females shared the same area, others showed no overlap at all. The introduced sisters W4 and W5 were trapped at the same location: underneath a small outbuilding, which presumably protects underlying nest sites from rain. Mice occupied this location throughout the entire study and we considered it as the most optimal nest site of the enclosure. The other introduced females W1–W3 settled at different places. Three daughters were recruited. W3.1 took over the area of her mother W3 after her mother disappeared/died. W4.1 and W4.2 stayed as well in the area of their mother and W4.1 reproduced in parallel with her mother although no weaned offsprings were trapped (Fig. 2). Only W4.2 survived the winter and started reproduction in 1998. Some of her daughters resettled and reproduced in different areas (W4.2.1 and W4.2.2) whereas some stayed at the same location but did not reproduce parallel with their mother (W4.2.3, W4.2.4, W4.2.5). Introduced females (W6–W9) either disappeared immediately after release (W6, W7) or showed partially overlapping areas with resident females but were never trapped around the optimal nest site (Fig. 3). Only three females survived to 1999: W4.2.4, W4.2.5 and W8; W4.2.4 remained at the optimal site, W8 remained at her territory and W4.2.5 moved to a new location to breed. W10 appeared as an immigrant female from the wild (Fig. 4).

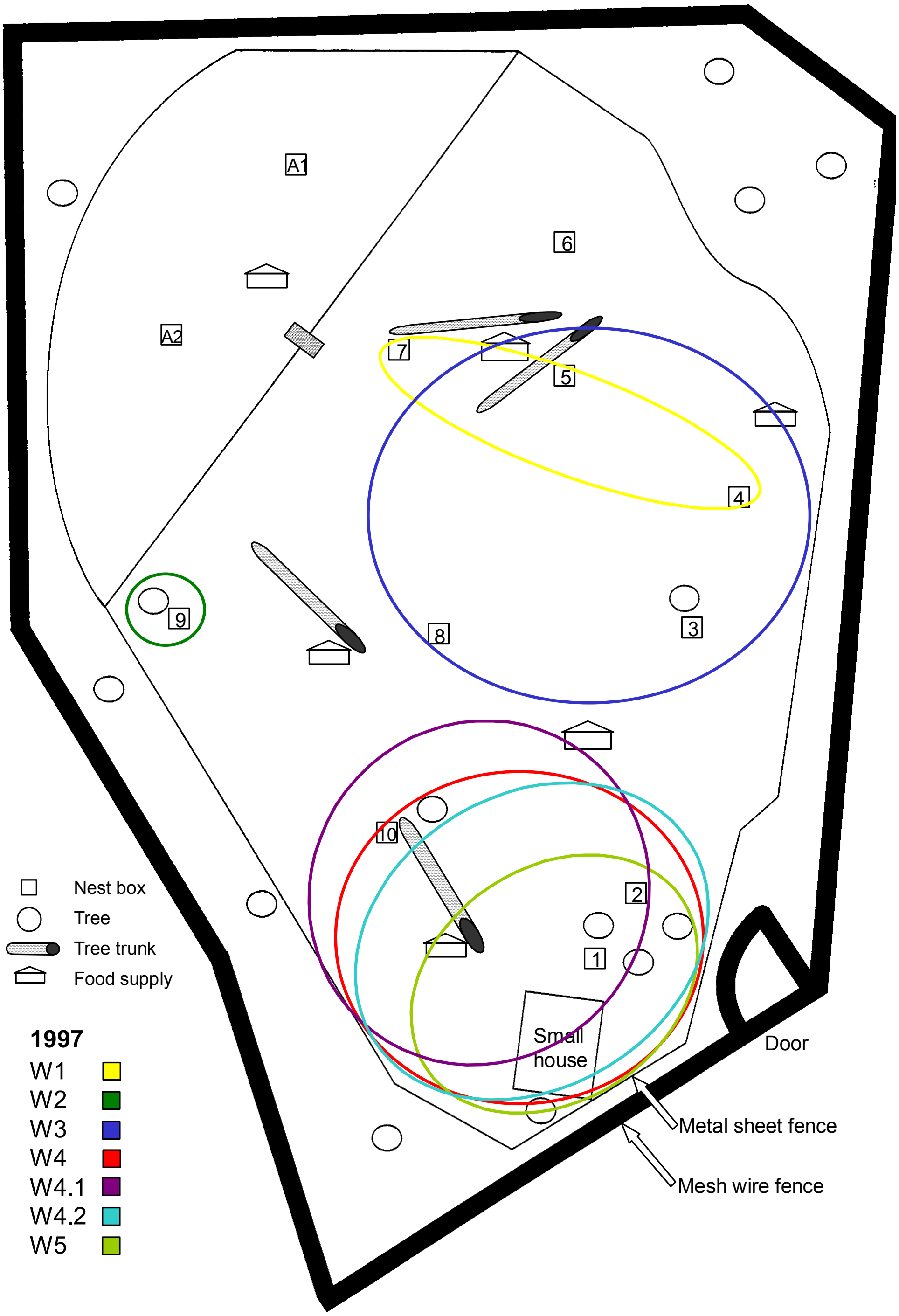

Fig. 2. The area of most frequent trapping of each resident female in 1997 in the enclosure indicated by colored circles.

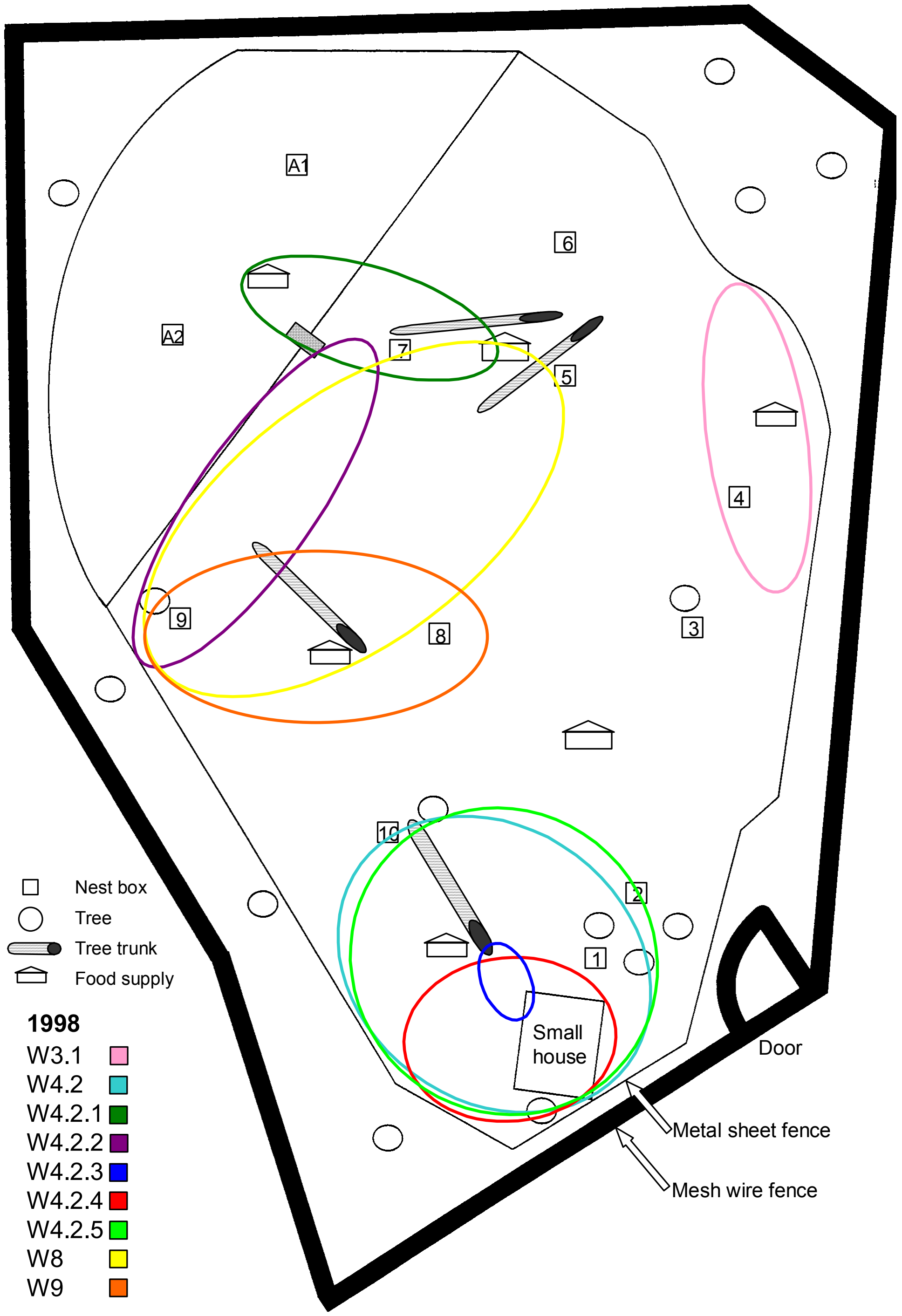

Fig. 3. The area of most frequent trapping of each resident female in 1998 in the enclosure indicated by colored circles.

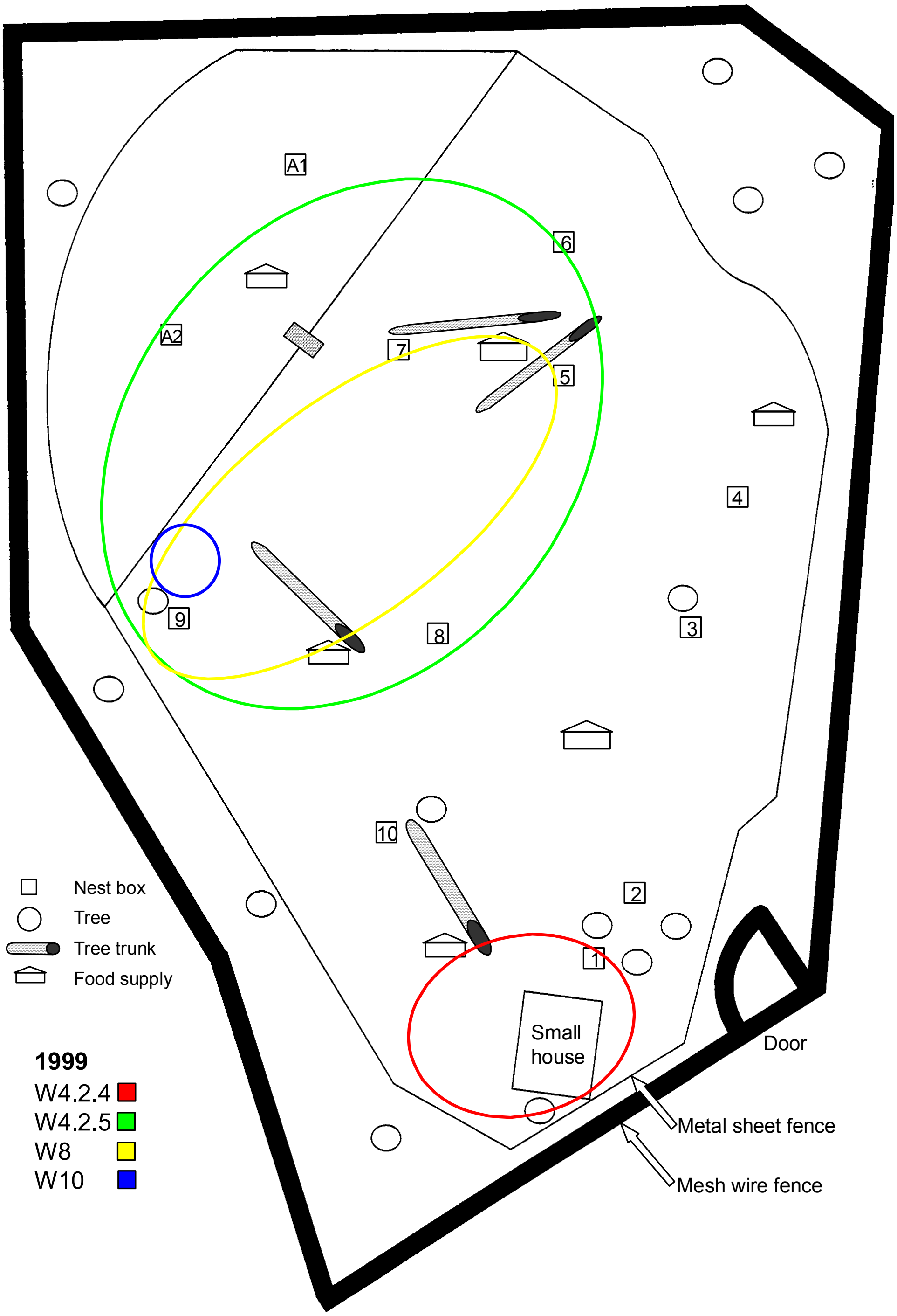

Fig. 4. The area of most frequent trapping of each resident female in 1999 in the enclosure indicated by colored circles.

Discussion

The structure of the investigated population in the enclosure was comparable with already known population dynamics in wood mice (Bergstedt, 1965; Flowerdew, 1972; Flowerdew, 1974; Montgomery et al., 1991; Canova et al., 1994; Schulte, 1997); low numbers in spring and summer and increasing numbers in late summer, autumn and winter. Deviations from the typical cycle could be correlated with weather conditions and/or food availability (Bengtson et al., 1989; Montgomery et al., 1991; Flowerdew, 2000; Flowerdew & Ellwood, 2001). Because food was offered *ad libitum,* weather conditions were regarded as the influencing factor. The availability of dry nest places therefore seems to have a strong impact on wood mice reproductive success.

Winter reproduction has been found in wood mice under good food conditions (Diaz et al., 1993). In the lab, wood mice breed throughout the year (Musolf, personal observation). However, there temperature and light conditions stay constant. The fact that in our population no winter reproduction is found could be due to insufficient food or nest site availability. If winter reproduction is genetically determined, the breeding lines of our lab might be randomly picked from non-winter outdoor breeders.

With the microsatellite analyses a high degree of multiple paternity was found in the enclosure population. Multiple paternity is recorded more and more often in natural populations as well in small mammals like deer mice (Birdsall & Nash, 1973), rats (McClintock et al., 1982), shrews (Stockley et al., 1993), meadow voles (Berteaux et al., 1999), and in different species of ground squirrels (Boellstorff et al., 1994; Foltz & Schwagmeyer, 1989; Hanken & Sherman, 1981; Hoogland, 1998; Murie, 1995). Baker et al. (1999) recorded multiple paternity in three out of 6 litters from wild trapped wood mouse females and Bartmann and Gerlach (2001) detected 85% multiple sired litters for wood mice in a large cage system. The mating system in wood mice could therefore considered as promiscuous, females mate with several males within one estrous period and males try to access as many females as possible (Bartmann & Gerlach, 2001). Infanticide could be prevented by confusing paternity with multiple mating of the females (Agrell et al., 1998). In a lab experiment we observed that wood mice females mate more often multiple times in the presence of infanticidal behavior of males and these multiply-sired litters survived better than single sired litters (Chapter 4). In the wild, where males and females have overlapping home ranges (Lambin & Krebs, 1991b; McShea & Madison, 1984; Wilson et al., 1992) and females have contact to several males, multiple mating seems to be a good strategy to prevent pups from infanticide.

It has been found that multiple mating may also be a means of inbreeding avoidance in shrews (Stockley et al., 1993). In our enclosure, often related wood mice males were around (33–64% of resident males were related to a least one of the resident females) and inbreeding took place three times. In two-third of the cases, females mated multiple times so we conclude that inbreeding avoidance could a possible advantage of multiple mating in wood mice as well.

Female body weight had no influence on the number of offspring they produced. This is in accordance with the study of Bartmann & Gerlach (2001) but it is in contrast to studies on other species, for example in house mice, where larger females have higher reproductive success (Gerlach, 1990; Gerlach, 1996). In our study the length of a wood mouse female's stay in the enclosure significantly influenced reproductive success. The longer a female was present the more offspring she reproduced. Therefore for wood mouse females it is necessary to have an established territory to be more successfully in breeding and weaning offspring. In *Peromyscus maniculatus borealis* the habitat of the nest site appeared to be important for the reproductive success of females as well (Sharpe & Millar, 1991). The stay in the enclosure for wood mice females could be connected with the availability of sufficient nest sites. In offering artificial nest boxes we tried to increase the availability of nest sites, but although these boxes were visited often they were never used for breeding. Obviously successful nest sites have additional criteria, which the artificial boxes did not fulfill. Maybe owning a good nest site not only improves the reproductive success but the survival of mother herself.

In contrast, there was no correlation between the length of a male's stay in the enclosure and the number of sired offspring. But heavier males were more successful than lighter males. Weight often corresponds with high social rank in most small mammals (Wolff, 1985b; Horne & Ylönen, 1998; Qvarnström & Forsgren, 1998) and is generally agreed that dominant animals gain higher access to mates, which causes a disproportionate mating success among dominant males (Dewsbury, 1982b). However, female wood mice did not choose their mates according to body weight or dominance (Chapter 2). In meadow voles (*Microtus pennsylvanicus*) it was also found that body mass played only a secondary role in driving female preferences (Berteaux et al., 1999). The higher reproductive success of heavier males may be related to ejaculate size, with larger males producing larger ejaculates (Gage et al., 1995; Simmons et al., 1996) or a higher frequency of copulating (McClintock et al., 1982) both transferring a larger number of sperm, which is the important determinant of success during sperm competition in mammals (Ginsberg & Huck, 1989).

In this study a significant higher variance in reproductive success was found in wood mouse females. This did not correspond with the finding of equal individual reproductive success of wood mice in a large cage experiment (Bartmann & Gerlach, 2001) and the expectation of equal variance in promiscuous mating systems. But wood mice are promiscuous as also indicated by the occurrence of multiple paternities in this study. No sex-dimorphism was found; both sexes did not differ significantly in body weight, which is also a hint for a monogamous or promiscuous mating system. A possible explanation may be provided by nest site availability as the most important factor influencing female reproductive success whereas the male's reproductive success is independent of these resources and therefore moderately divided among them.

One matriline was most successful in the enclosure. We suggest two possible explanations for this: either these females are the best adapted for the environment they have to deal with in the enclosure compared to the other breeding lines from our lab or initial territory selection was random and the one with the best territory of the enclosure became the most successful one. The daughters inherited the territory and therefore had also a reproductive advantage over the other females inhabiting minor territories in the enclosure.

Territory quality is the best predictor of the group's reproductive success as has been shown in Tamarin native hens (*Gallinula morterii*) (Goldizen et al., 1998). Expansion of family lines was dependent on the number of high-quality territories nearby (Putland & Goldizen, 2001). Koenig and Mumme (Koenig & Mumme, 1987) analyzed the survivorship of lineages in the acorn woodpecker. They found that lineages persisted longer when on higher-quality territories and concluded that territory inheritance may be an important benefit of group living in that species. Actually we found territory inheritance in female wood mice several times in our enclosure. Four females inherited the nest site of their mother, one of them bred parallel without any success, and three were tolerated in their mothers' territory but did not breed.

We did not find strong evidence for a kind of helper system. Wood mice females were able to discriminate between own pups and pups of mothers, sisters and unrelated females (Gerlach & Bartmann, 2002) relating their nursing effort in a communal nest strongly to the age of their own pups and reducing it almost entirely when all own pups were killed. It is not known if non-breeding wood mice staying at their familiar nest site help their mothers with the new litter. One possible aspect could be thermoregulation or nest defense while the mother is away.

Communal nesting and breeding alone is not advantageous for wood mice females (Gerlach & Bartmann, 2002) but under nest site limitation a mother should accept a daughter who has no other chances for reproduction. Under harsh ecological conditions this makes it necessary to save time and energy even communal breeding sisters would gain benefits compared to singly breeding females. Maybe this has been the basis for the communal breeding of the sister pair W4 / W5 at the start of the experiment. However, we only could determine the communal use of a location and not directly the communal breeding. Increase in survivorship and higher reproductive success gives an indication why it could be better for females to be philopatric and carry the cost of reproductive suppression in the beginning of their life. In yellow-bellied marmots (*Marmota flaviventris*) (Armitage & Schwartz, 2000) the mortality of dispersing yearlings is significantly higher than that of yearling residents.

Montgomery and colleagues (1997) determined that resident females inhibit reproductive activity in immigrant wood mouse females. In the investigated enclosure 4 females were released at different times where other females (3–6) were already established. While two of the introduced females successfully reproduced, their reproductive output was much lower than that of the resident females (2 compared to 3–24 offspring) and they stayed just a month in the enclosure. The two immigrant females from the wild did not establish and breed at all. Three of 15 immigrant males were able to settle in the enclosure and were as successful in siring offspring (4–6) as resident males. In a study on an open grid on meadow voles (*Microtus pennsylvanicus*) Pugh and Tamarin (1991) determined that immigrant males tended to live longer than resident males but this did not result in significantly greater reproductive success of immigrants. Immigrants appear to be as fit as residents and they can be as successful as the residents if they can establish themselves in their new home range. Clearly in this study the immigrant females had no chance to establish in good territories because these were already occupied by the breeding females and their daughters.

To summarize our results: We found strong evidence that female daughters benefit by staying in the territory of their mothers by inheriting their nest site resulting in higher reproductive success in the wild. The nest site availability was the most important factor influencing reproductive success of females indicated by a correlation of the reproductive success with the stay in the enclosure.

The high occurrence of multiple paternity in wood mice litters confirms the promiscuous mating system.

GENERAL DISCUSSION

The major findings of this thesis can be summarized as follows. The primary focus of this work has been on mate choice in female wood mice (*Apodemus sylvaticus*). For the first time, I could show that female wood mice are able to recognize kin and actively avoid inbreeding. Furthermore, females are surprisingly indiscriminate in choosing mates of different quality such as body weight, dominance and, very unusually, MHC genotype. Multiple mating occurs frequently and I showed that females use this behavior as a counter-strategy against infanticidal males. Females generally avoid each other, indicating competition for resources. Under limited resources (measured as nest site availability), I found a novelty in female wood mice social behavior: inheritance of the mother's territory.

Female mate choice in wood mice

Kin recognition and inbreeding avoidance?

Understanding recognition systems is important to developing and testing hypotheses about such diverse topics in evolutionary biology as mate choice and nepotism. Kin recognition, the ability of an organism to determine genetically related individuals, is an important ability, especially with regard to social behavior. While mating with kin should be avoided to prevent inbreeding and the resulting harmful effects (Ralls et al., 1986; Charlesworth & Charlesworth, 1987) kin should be sought out as recipients of assistance to increase the donor's inclusive fitness (Hamilton, 1964).

In Chapter 1, I studied the ability of wood mouse females to identify kin in mate choice decisions. Results demonstrated that the estrus stage of the female had a significant impact on her behavior, indicating that actually a distinct mate choice behavior exists. Estrous females preferred odors of unfamiliar unrelated males to the odor of brothers with whom they had no previous contact. Different recognition mechanisms exist and all are based on learning (Tang-Martinez, 2001). Recognition by association and by phenotype matching are the two most widely accepted mechanisms of kin discrimination (Holmes & Sherman, 1983). On the basis of my result it appears that familiarity with the male was not the crucial factor in these tests of odor preference. These data suggest that wood mouse females might recognize unfamiliar kin using phenotype matching. From the odor preference shown in this study, I would predict that, given a choice, females should avoid mating with brothers and perhaps other related individuals. In addition, a form of inbreeding barrier or inbreeding avoidance between siblings was suggested by the fact that littermates, when kept together after weaning, reproduced in only 5–10% of all cases after reaching adulthood whereas almost 100% of unfamiliar unrelated couples bred (Musolf, personal observation).

Phenotype matching may be based on comparisons between the odor of novel males and either the female's own phenotypic odor template or that of familial conspecifics (littermates or mothers). Odor templates might be formed by cues from the major histocompatibility complex (MHC) gene products (Apanius et al., 1997; Jordan & Bruford, 1998). Alternatively, rodent urine also contains a class of proteins, termed major urinary proteins (MUPs) that bind and release small volatile pheromones. In wild house mice (*Mus domesticus*) the combinatorial diversity of expression of MUPs might be as great as that of MHC, and, with protein concentrations a million times higher than MHC, MUPs might also play an important role in the individual recognition mechanism (Hurst et al., 2001).

My experiments did not differentiate between self-matching or familial imprinting in female mating decisions. The ability to self-match is controversial (see Alexander, 1991; Sherman et al., 1997). In the non-social, promiscuous golden hamster (*Mesocricetus auratus*) self-matching is used in mate choice decisions (Mateo & Johnston, 2000). Considering the high degree of multiply-sired litters and the promiscuous mating system in wood mice (Baker et al., 1999; Bartmann & Gerlach, 2001) self-matching would also be an appropriate mechanism for differentiation between individuals of different genetic relatedness in wood mice.

In less social species that are likely to encounter unfamiliar individuals that may or may not be their relatives, the discrimination between kin and non-kin based on genetic relatedness through comparison of a conspecific's phenotype with a known kin phenotype is assumed to be particularly useful (Halpin, 1991). To evaluate if sociality of a species plays a role in determining the mechanism involved in avoiding mating with relatives, I have compared these results with those of previous studies on female mate choice in rodents.

The role of familiarity versus phenotype matching can be determined by odor choice tests or by mate choice tests asking females to differentiate between familiar/unfamiliar and related/unrelated odors or individuals. In social rodents, two species (*Peromyscus leucopus* (Grau, 1982; Keane, 1990); *Mus domesticus* (Gilder & Slater, 1978; Egid & Lenington, 1985; Lenington & Egid, 1985; Winn & Vestal, 1986; Dewsbury, 1988; Potts et al., 1991) were shown to use phenotype matching while in five others, differentiation was based on familiarity (*Peromyscus maniculatus* (Hill, 1974), *Microtus ochrogaster* (Gavish et al., 1984; DeVries et al., 1997), *M. canicaudus* (Boyd & Blaustein, 1985), *M. mandarinus* (Fadao et al., 2000), *Heterocephalus glaber* (Clarke & Faulkes, 1999)). Exclusive use of familiarity could be shown in three species (*Microtus mandarinus* (Fadao et al., 2000), *M. canicaudus* (Boyd & Blaustein, 1985), *Heterocephalus glaber* (Clarke & Faulkes, 1999)), while only the house mouse was shown to use both mechanisms (Yamazaki et al., 1988).

Fewer studies are available for species with reduced sociality. In the golden hamster (*Mesocricetus auratus*) (Mateo & Johnston, 2000) and in the cactus mouse (*Peromyscus eremicus*) (Dewsbury, 1982a) females used both phenotype matching and familiarity, while meadow vole females (*Microtus pennsylvanicus*) (Salo & Dewsbury, 1995) and montane voles (*M. montanus*) (Berger et al., 1997), were shown to use familiarity while the role of relatedness was not studied. In contrast to previous studies, our results show that female wood mice use phenotype matching. Therefore, it appears that both mechanisms can be used in less social rodent species.

Social rodents generally use familiarity and not phenotype matching while both mechanisms seem to be used in less social species. This contradicts the assumption that phenotype matching should be more important in less social species. Obviously, sociality of a species *per se* does not allow a good prediction for which kin recognition mechanism is used in mate choice decisions.

It is becoming more and more evident that both mechanisms may co-exist and can be demonstrated under different experimental conditions (Kareem & Barnard, 1982; Hepper, 1983; Porter et al., 1983; Heth et al., 1998; Todrank et al., 1999). Some rodents use one recognition mechanism for mate choice; in social interactions they were able to use the other mechanism (Heth et al., 1998; Todrank et al., 1999). And a recent study has criticized these studies as that the discriminative tests are designed in a way that biases the result towards familiarity (Todrank & Heth, 2001).

It was clearly shown in this study that female wood mice were able to recognize their kin. With this important ability they were able avoid inbreeding even with unfamiliar related males.

Mate choice criteria

The avoidance of kin is just one component of mate choice decisions. Other expression of female preferences might be due to variations in male quality. Even if males do not provide any parental care, females still benefit from high quality males with regard to 'good genes' conferring greater fitness on the female's offspring (Møller & Alatalo, 1999). In Chapter 2, the influence of different male characteristics such as body weight and dominance rank on female preference was tested. Female wood mice did not prefer heavier males although they showed a slight tendency to choose the heavier of two males as weight difference between them increased. Dominance of one male over the other had also no influence on female choice. Although females avoided the combined odor of a pair, they did not differentiate between the odor of a solitary male and a male already paired with another female; therefore, they did not copy pairing behavior of another female. Finally, and in contrast, to previous studies on other small mammals (Desjardins et al., 1973), we did not find any relationship between body weight, social dominance, and urination patterns in male wood mice.

The lack of female preference for heavier and/or dominant males is surprising as female wood mice are capable to discriminate between different mating partners based on odor cues (Chapter 1) and small mammals females are often able to discriminate between males according to their social status and generally prefer dominant males as mating partners. Apps et al. (1988) and Novotny et al. (1990) showed that the quality of urinary odors of subordinate male house mice was influenced by hormonal changes resulting from long-term social subordination. Differences in urine quality from dominant and subordinate males can be detected by female house mice (Hurst, 1990; Rich & Hurst, 1998), bank voles (*Clethrionomys glareolus*) (Hoffmeyer, 1982; Horne & Ylönen, 1996), hamsters (*Mesocricetus auratus*) (White, 1986) and brown lemmings (*Lemmus trimucronatus*) (Huck et al., 1981; Huck & Banks, 1982). Shapiro and Dewsbury (Shapiro & Dewsbury, 1986) found female preference for dominant males as mates in the prairie vole (*Microtus ochrogaster*) based on odor contact alone and in real matings. However, females of the promiscuous montane vole (*M. montanus*) show no preference with regard to male dominance (Shapiro & Dewsbury, 1986) as has been found in meadow voles (*M. pennsylvanicus*) (Berteaux et al., 1999). Agrell (1997) found that female field voles (*M. agrestis*) preferred dominant males when density was low but showed no preference when male density was high, suggesting that female preference may be context rather than species dependent. Therefore, the mating system and the population density might influence female mate choice preferences also in wood mice?

In the current study, females avoided the odor of a pair, perhaps using odors to avoid nest sites potentially defended by other females, a pattern seen in house mice (Hurst & Nevison, 1994). Clearly, female wood mice can and do use at least some odor cues to differentiate between males including the subtle differences involved in determining relatedness.

A possible explanation is that, in contrast to other species of voles and mice, preference for heavier or dominant males is absent in female wood mice. This could also explain why male wood mice do not express differences in scent marking behavior according to their body weight or dominance rank known from male house mice (*Mus musculus*). House mice exhibit a clear rank-dependent urination pattern with increased marking by dominant males and decreased marking by subordinates (Desjardins et al., 1973).

The mating system might be the crucial factor in explaining differences in female preferences. House mice live in family groups dominated by a single male who sires most of the offspring. Even the odor of a foreign male can cause fetal resorption or abortion (Bruce, 1960). In contrast, female wood mice are solitary and mate with several males within one estrus period both in the field and in enclosures (Baker et al., 1999; Bartmann & Gerlach, 2001). Faced with multiple males, the female may not be able to afford herself the luxury of selecting one partner when non-mated males are infanticidal (Chapter 3). Thus, avoidance of infanticide might outweigh the benefits of mating with heavier or dominant males exclusively. While females do select against closely related males (Chapter 1), it is not known if such closely related males are less infanticidal, a notion that would be suggested by consideration of inclusive fitness.

The relationship between relatedness and infanticide is an interesting remaining question. It also remains to be analyzed if females discriminate against clearly inferior males, e.g. recognizing strains of pathogens and parasites. Recognizing these attributes might be under stronger selection pressure than preferring males of higher body weight or dominance. In this case, females are assumed to mate with males with complementary alleles at crucial immune-coding loci.

The results of these odor choice tests indicate that the earlier results that heavier males had a higher reproductive success than lighter males are not based on female odor preference for heavier males. In promiscuous mating systems, the higher reproductive success of heavier males might be caused by factors different from or in addition to female choice. Heavier males could have more or better sperm. They could also have access to females for a longer period or at the most propitious time (Schwagmeyer & Parker, 1987) as shown in rats (McClintock et al., 1982) and bats (Hosken, 1998) when timing of insemination is important in determining fertilization success. In meadow voles (*Microtus pennsylvanicus*), where females also show no preference for heavier males, male body mass has a significant influence on the number of copulations performed by males (Berteaux et al., 1999). Here, females did not distribute copulations evenly among males, preferring the heavier male with increasing difference between the body masses between the males.

Mate choice and MHC

Further mate criteria are provided by variation at MHC loci. Assuming that immunocompetence is enhanced by variable MHC loci and kin share a high frequency alleles at these high polymorphic loci, MHC-disassortative mating is predicted as a means to increase the fitness of the progeny and avoid inbreeding. In Chapter 3, female mate choice in wood mice was investigated with respect to MHC genotype in laboratory odor tests and in an enclosure population. Two MHC loci have been analyzed, SiSo1&2 and RT1.*Ba,* both with low levels of variation in wood mice compared to other species (Slade et al., 1993; Seddon & Baverstock, 1999). Astonishingly, in this study, females did not show a preference for the odor of a dissimilar or a similar male. Heterozygosity of the male also had no influence on female decisions. Furthermore, in the enclosure, females chose their mates regardless of the number of alleles a male had. In summary, neither the females in the odor test nor the females in the enclosure exhibited a MHC-dependent preference. This is in contrast to findings in house mice (*Mus domesticus*) and humans, who mate disassortatively with respect to MHC loci and demonstrate no general preference of avoidance of specific MHC haplotypes (Egid & Brown, 1989; Potts et al., 1991; Wedekind et al., 1995; Ober et al., 1997). Non-disassortative mating often correlates with frequent spontaneous abortions or prolonged intergestational intervals in mice, humans and pigtailed macaques (Warner et al., 1991; Jin et al., 1995; Ober, 1995; Knapp et al., 1996). The results of our study may be explained by MHC-independent mate choice. Female wood mice are indiscriminate in choosing mates of different quality (Chapter 2), including the dominance rank of the male. Instead, they mate multiple times with all males in their neighborhood confusing paternity and protecting their litters from infanticidal males (Chapter 4). This effect clearly has a stronger impact on female mating behavior than variation in male traits, possibly including variation in MHC genotypes. While the lack of preference for MHC genotypes might also be due to an experimental error in the determination of different MHC alleles caused either by the presence of null alleles or the insufficiency of detecting all alleles with the SSCP method, the consistency of results across both MHC loci warrants further investigations.

Interesting insights are gained from a comparison of eight neutral markers (microsatellites) with the genes for MHC. Whereas all neutral markers are in Hardy-Weinberg equilibrium, the two MHC loci exhibit significant heterozygote deficiencies. The causes for this effect should be investigated further.

Focusing on the distinction between similar and dissimilar MHC genotypes may not be sufficient when parasite resistance is important. In these situations, females should choose partners that maximize the number of different MHC alleles in their offspring (Penn & Potts, 1999). In female sticklebacks (*Gasterosteus aculeatus*), males with many alleles were chosen in mate choice decisions whereas the similarity of the male genotype to the female had no influence (Reusch et al., 2001). These results were based on the analysis of 6 different MHC class-IIB loci. The reduced variability and the low number of different loci used in our study on wood mice could contribute to the lack of preference for males with few or many different MHC alleles. Further MHC loci should be analyzed to become better insight in MHC dependent mating patterns.

Multiple mating: a counter-strategy to infanticide?

Infanticide among mammals is a widespread phenomenon with no general explanation. In Chapter 4 of this study, I focus on the behavioral strategies used by females to avoid major losses in reproductive success by males committing infanticide. There is a clear reproductive advantage for male wood mice to kill strange offspring because following infanticide the female's gestation duration is significantly shortened (from 27.2 days to 23.6 days; ANOVA, $F = 34.6$, $P = 0.0001$). Therefore, infanticidal males can potentially mate more quickly with the mother of the killed pups than they would have otherwise, and hence increase their reproductive success over their short life span of just a few months. Males are apparently not able to differentiate between their own pups and those sired by other males. To avoid killing their own pups they accept the whole litter. In the presence of a mixed litter, the level of infanticide is significantly reduced from 53% to 11% (Fishers exact test, $chi^2 = 5.06$, $P = 0.031$).

Female counter strategies to infanticide include aggression, female choice of dominant males, and promiscuity to confuse paternity as defense against males. Counter-strategies to infanticide not only have the potential to affect the behavior of individuals (e.g. aggression, spacing and mate choice), but may also have consequences for the shaping of mammalian mating systems.

As has been already shown in Chapter 2 wood mouse females do not prefer dominant males. Dominant males are considered better defenders of territory, nest site and/or offspring (Huck et al., 1982; Reich et al., 1982; vom Saal & Howard, 1982; Nelson, 1994). In wood mice, males and females neither cohabit exclusively the same territory nor is the male involved in parental care or the defense of the female's nest. Therefore, the dominance rank of a male *per se* does not provide any benefit for the female. In contrast, the female potentially has to deal with several males visiting her home range (Tew & Macdonald, 1994). Female mammals often use aggression to keep males away from their nest sites (Elwood & Ostermeyer, 1984; vom Saal, 1984; Montgomery & Gurnell, 1985; Wolff, 1985a). However, intersexual territoriality is generally rare in mammals and wood mouse females might be not effective in keeping males from their nest sites because they are slightly smaller than males. Even if females are effective in limiting male access to their nest, females with post-partum estrus (the estrus immediately after have given birth) must associate with at least one potentially infanticidal male during the time when her pups are most vulnerable.

The occurrence of infanticide in the lab where food and nest sites are not restricted and therefore no reason exists for the female to commit infanticide herself suggests that wood mouse females are not able to protect their litter entirely from intruding males as has also been shown by Wilson (1993a)

Therefore females must rely on an alternative counter-strategy to infanticide. A common feature in species in which male infanticide is prevalent is for females to mate promiscuously with all males in the vicinity to confuse paternity (O'Connell & Cowlishaw, 1994; Wrangham, 1997). Males that have copulated are generally inhibited from killing pups for the time period in which their young would be vulnerable to infanticide. In white-footed mice (*Peromyscus leucopus*), it has been shown that males were inhibited from killing pups for about 35 to 40 days after copulation, the time equal to gestation and nursing for this species (Cicirello & Wolff, 1990). Similar results were found for laboratory (vom Saal & Howard, 1982) and wild mice (Soroker & Terkel, 1988), and Mongolian gerbils (*Meriones unguiculatus*) (Elwood, 1977).

The use of multiple mating as a mechanism to generate uncertainty of paternity to deter infanticide has been proposed for many species (see Agrell et al., 1998), but direct support for this hypothesis is limited. Chapter 4 provided the first evidence that wood mouse females actually use multiple mating to reduce their risk of exposure to male infanticide. Single sired litters were more often the victim of infanticide than multiply-sired litters and, after the occurrence of infanticide, females were significantly more likely to mate with multiple males than if their first litter survived. Polyandry appears to be an active female strategy to avoid infanticide.

The avoidance of infanticide helps to explain the evolution of female promiscuous matings in many species. Female multiple mating may also serve as a means of protecting females and their offspring from the attacks of cuckolded males. Whatever the case, avoidance of infanticide is an important driving force in the evolution of many mating systems and should be the topic of further investigations.

Microsatellite markers

Microsatellite markers provide a powerful alternative for investigations of population structure compared to previous methods such as allozyme analyses or other DNA analyses such as multilocus DNA fingerprinting, RAPD-PCR techniques (rapid amplified polymorphic DNA), and RFLP (restriction fragment length polymorphism). Even when allozyme variation is low, microsatellite loci may be highly polymorphic (Hughes & Queller, 1993). Other advantages to this PCR-based approach are the slight amounts of DNA needed for the analysis, the low size of the microsatellite loci (rarely larger than 300 bp) and reproducible and unambiguous results because a single diploid individual has at most two alleles at a single microsatellite locus. Microsatellite loci often provide a higher degree of resolution for elucidating relationships between closely related taxa (Bowcock et al., 1994) and within familial groups (Amos et al., 1993) and are particularly useful for evaluating mating success.

In this study, 6 new microsatellites for the wood mouse (*Apodemus sylvaticus*) were found. Observed heterozygosity ranged from 0.74 to 0.97 and number of alleles per locus was relatively high with 7 to 15 different alleles. No linkage disequilibrium was detected; all loci are independent of each other. I have chosen 5 of them on the basis of easy and clear readability of different alleles on the gel. Combined with three microsatellites previously developed for the yellow-necked mouse (*Apodemus flavicollis*) (Gockel et al., 1997) that also amplify in wood mice, I used a set of 8 highly polymorphic microsatellites that made it possible to resolve family structures and/or paternity of offspring even when test animals were closely related.

Social behavior among female wood mice in the wild

Chapter 6, details a three-year observational study of a semi-natural population of wood mice. Date concerning population level and fluctuations throughout the year showed no deviation from natural populations, confirming the application of the gained insights of this study to natural wood mice behavior.

With the microsatellite analyses a high degree of multiple paternity was found in the enclosure population. Multiple paternity is recorded more and more often in natural populations as well in small mammals (Birdsall & Nash, 1973; Hanken & Sherman, 1981; McClintock et al., 1982; Foltz & Schwagmeyer, 1989; Stockley et al., 1993; Boellstorff et al., 1994; Murie, 1995; Hoogland, 1998; Berteaux et al., 1999). Baker et al. (1999) recorded multiple paternities in three out of 6 litters from wild-trapped wood mouse females and Bartmann and Gerlach (2001) found that 85% of wood mice litters were multiply sired in a large cage system. The results from this study confirm the previous findings and additionally make clear that multiple mating seems to be a general behavioral pattern of wood mice and not a cage artifact or a random effect in the wild. The mating system of wood mice should be considered as promiscuous. Females mate with several males within one estrus period and males try to access as many females as possible. Advantages of this behavior such as infanticide avoidance are discussed above.

Female body weight in the enclosure had no influence on the number of offspring they produced, which is in accordance with the study of Bartmann & Gerlach (2001). These results are in contrast to other studies, for example in house mice, where larger females had higher reproductive success (Gerlach, 1990; Gerlach, 1996). The important factor for wood mice female reproduction is the period of stay in the enclosure. The longer a female was present in the enclosure the more offspring she reproduced. Therefore, for wood mouse females it appears necessary to become established and own a territory in order to be more successful in breeding and weaning offspring. In *Peromyscus maniculatus borealis*, the habitat of the nest site appeared to be important for the reproductive success of females as well (Sharpe & Millar, 1991). The stay in the enclosure for wood mice females could be connected with the availability of sufficient nest sites. Maybe owning a good nest site not only improves the reproductive success but the survival of the female herself.

Nest site availability appeared to be the crucial factor for female reproductive success. Food was offered *ad libitum* and therefore could not be the restricting element. Throughout the whole experiment only four females bred parallel although more females were living in the enclosure at the same time. This indicates that breeding is somehow restricted and might be due to limited nesting space and/or reproductive suppression by the established females. That wood mouse females are capable to suppress reproduction in other females has been shown in a previous study (Montgomery et al., 1997).

Which opportunities does a wood mouse female have when she reaches sexual maturity at an age of approximately 8 weeks and with a life expectancy of, on average, 6 to 8 months? Either she conquers an own territory and as a result she is able to reproduce or she stays in her mother's territory, possibly suffers from reproductive suppression, or breeds communally with the mother or helps the mother with breeding and finally inherits the territory. Solitary breeding offers the best fitness opportunities and therefore should be preferred (Gerlach & Bartmann, 2002). However, if all available sites are occupied, a female might improve her fitness by breeding in the same territory as her mother, although her reproductive success is lower than solitarily breeding females (Gerlach & Bartmann, 2002). In the enclosure a mother and daughter bred parallel at the same location on a single occasion, and, in this case the daughter produced no weaned offspring. Therefore I assume that communal breeding is not a common pattern in wood mouse females.

In this study on wood mice females a further advantage of staying with the mother has been identified: territory inheritance. Females of one matriline were significantly more successful than female of other matrilines. The founder of this matriline became established in the best territory of the enclosure and daughters and granddaughters who inherited this territory also had exceptionally reproductive success. Staying in a familiar territory, although it might be combined temporarily with a decrease in reproduction, offers a successful alternative if nest sites are limited.

Conclusions

Wood mice live semi-socially in open habitats such as the edges of the forest, clearings in deciduous and mixed forest, garden and parks (Niethammer & Krapp, 1982). Compared to house mice, whose environment in general is restricted to a certain area (e.g. storehouse, barn, attic, cereal ricks) (Berry, 1981) which can be better controlled, wood mice are not able to avoid other individuals crossing their territories; males might not have the capability to guard their mates and prevent mating with other males entirely, and females might not be able to defend their nest sites from disturbing conspecifics. This is especially important at high population densities. Schulte (Schulte, 1997) found peak densities with 80 individuals/ha in the forest. Other species (bank voles, yellow-necked mice) cohabit the same area supplementary reaching similar densities and competing also for food and nest sites. In such an environment males are not able to provide material benefits such as exclusive food resources or nest sites and, in addition, females have to deal with intruding conspecifics and other species that might be infanticidal.

To summarize the results from the studies of this thesis, the following picture of wood mice behavior can be drawn. Wood mouse males follow the widespread pattern to mate with any available female to improve their reproductive success. They also gain reproductive increase by killing offspring of other males. Infanticide by males provides a strong selection pressure on the development of counter-strategies by females. In wood mice, female promiscuity seems to be a successful tool to protect offspring by confusing paternity. Males are not able to identify foreign offspring in a litter they have only partially sired and therefore accept the whole litter.

The lack of female preference for distinct male traits (e.g. body weight, dominance, MHC genotype) and the lack of support for signal of male dominance and body weight in urination pattern can be explained against this background of promiscuous mating. Females will mate with all males in the vicinity if non-mating males are infanticidal. Therefore no selection pressure is provided by female mate choice on the evolution of specific male characteristics and, for males, it is of no use to advertise dominance ranks in any way if they are not a target for female choice, especially, when signaling is costly (Gosling et al., 2000). However, male traits such as body weight might nevertheless be correlated with other important characteristics such as the production of more or better sperm (sperm competition), or the access to females for a longer period or at the most propitious time, which might explain why it is that heavier males sire more offspring even without female choice for heavier males.

Selection against inbreeding is a strong driving force in the evolution of female mate choice. In accordance with the promiscuous mating system, the semi-social behavior and the resulting similar degree of relatedness within a litter of half-sibs and between litters of different females sired of the same group of fathers, a kin recognition system based only on familiarity would not be adequate. In wood mouse females, the use of phenotype matching as kin recognition mechanism has been shown and kin have been avoided in mate choice decisions. Promiscuity itself also provides further help to reduce inbreeding as has been shown in common shrews (Stockley et al., 1993) and might be also helpful in wood mice.

Besides mate choice decisions, another aspect influences female reproductive success. This study revealed that the property of a nest site of good quality is very important for the female’s fitness. Females prefer to breed solitarily which was also supported by the odor tests in Chapter 2 where females avoid the odor of another female. At the same time, if space is limited for daughters, the opportunity to remain in their natal territory and hopefully inherit it, provides an alternative strategy compared to never having a chance to breed.

The behavioral patterns of this semi-social woodland species highlight the flexibility of rodents in adapting to different environmental conditions. Wood mice lack stable family and mating bonds and/or territory defense but are equipped with sufficient behavioral mechanisms to avoid inbreeding and increase reproductive success. This may increase their capacity for colonization and may partially explain their widespread distribution.

BIBLIOGRAPHY

Agrell, J. 1997. Experimental testing of female mating strategies in microtine rodents. In: *Abstracts of the Seventh International Theriological Congress*, pp. 5. Acapulco, Mexico.

Agrell, J., Wolff, J. O. & Ylönen, H. 1998. Counter-strategies to infanticide in mammals: Costs and consequences. *Oikos*, **83,** 507–517.

Ågren, G. 1984. Incest avoidance and bonding between siblings in gerbils. *Behavioral Ecology and Sociobiology*, **14,** 161–169.

Alexander, R. D. 1991. Social learning and kin recognition. An addendum and reply to Sherman. *Ethology and Sociobiology*, **12,** 387–399.

Amos, B., Schlötterer, C. & Tautz, D. 1993. Social structure of pilot whales by analytical DNA profiling. *Science*, **260,** 670–672.

Andersson, M. 1994. *Sexual selection.* New Jersey: Princeton University Press.

Apanius, V., Penn, D. J., Slev, P. R., Ruff, L. R. & Potts, W. K. 1997. The nature of selection on the major histocompatibility complex. *Critical Reviews in Immunology*, **17,** 179–224.

Apps, P. J., Rasa, A. & Viljoen, H. W. 1988. Quantitative chromatographic profiling of odours associated with dominance in male laboratory mice. *Aggressive Behaviour*, **14,** 451–461.

Armitage, K. B. 1999. Evolution of sociality in marmots. *Journal of Mammalogy*, **80,** 1–10.

Armitage, K. B. & Schwartz, O. A. 2000. Social enhancement of fitness in yellow-bellied marmots. *Proceedings of the National Academy of Sciences of the United States of America*, **97,** 12149–12152.

Baker, R. J., Makova, K. D. & Chesser, R. K. 1999. Microsatellites indicate a high frequency of multiple paternity in *Apodemus* (Rodentia). *Molecular Ecology*, **8,** 107–111.

Balshine-Earn, S., Neat, F. C., Reid, H. & Taborsky, M. 1998. Paying to stay or paying to breed? Field evidence for direct benefits of helping behavior in a cooperatively breeding fish. *Behavioral Ecology*, **9,** 432–438.

Barnard, C. J. 1990. Kin recognition: problems, prospects and the evolution of discrimination systems. *Advances in the Study of Behavior*, **19,** 29–81.

Barnard, C. J. & Fitzsimons, J. 1988. Kin recognition and mate choice in mice: the effect of kinship, familiarity and social interference on intersexual interaction. *Animal Behaviour*, **36,** 1078–1090.

Barnard, C. J. & Fitzsimons, J. 1989. Kin recognition and mate choice in mice: fitness consequences of mating with kin. *Animal Behaviour*, **38,** 35–40.

Bartmann, S. & Gerlach, G. 2001. Multiple paternity and similar variance in reproductive success of male and female wood mice (*Apodemus sylvaticus*) housed in an enclosure. *Ethology*, **107,** 889–899.

Basolo, A. L. 1990. Female preference for male sword length in the green swordtail *Xiphophorus helleri* Pisces Poeciliidae. *Animal Behaviour*, **40,** 332–338.

Beauchamp, G. K., Yamazaki, K., Bard, J. & Boyse, E. A. 1988. Preweaning experience in the control of mating preferences by genes of the major histocompatibility complex of the mouse. *Behavior Genetics*, **18,** 537–547.

Belkhir, K., Borsa, P., Goudet, J., Chikhi, L. & Bonhomme, F. 1997. Genetix v. 3.0, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome et Populations, CNRS UPR 9060, Université Montpellier 2, Montpellier.

Bengtson, S. A., Nilsson, A. & Rundgren, S. 1989. Unforeseen disruption of wood mouse population dynamics after food reduction: a field experiment. *Oikos*, **56,** 379–385.

Benhamou, S. 1990. An analysis of movements of the wood mouse *Apodemus sylvaticus* in its home range. *Behavioural Processes*, **22,** 235–250.

Bensch, S. & Hasselquist, D. 1992. Evidence for active female choice in a polygynous warbler. *Animal Behaviour*, **44,** 301–311.

Berger, J. & Cunningham, C. 1987. Influence of familiarity on frequency of inbreeding in wild horses. *Evolution*, **41,** 229–231.

Berger, P. J., Negus, N. C. & Day, M. 1997. Recognition of kin and avoidance of inbreeding in the montane vole, *Microtus montanus*. *Journal of Mammalogy*, **78,** 1182–1186.

Bergstedt, B. 1965. Distribution, reproduction, growth and dynamics of the rodent species *Clethrionomys glareolus* (Schreber), *Apodemus flavicollis* (Melchior) and *Apodemus sylvaticus* (Linné) in southern Sweden. *Oikos*, **16,** 132–160.

Berry, R. J. 1981. *Biology of the house mouse.* Oxford: Clarendon Press.

Berteaux, D., Bety, J., Rengifo, E. & Bergeron, J.-M. 1999. Multiple paternity in meadow voles (*Microtus pennsylvanicus*): investigating the role of the female. *Behavioral Ecology and Sociobiology*, **45,** 283–291.

Birdsall, D. A. & Nash, D. 1973. Occurrence of successfull multiple insemination of females in natural populations of deer mice (*Peromyscus maniculatus*). *Evolution*, **27,** 106–110.

Birkhead, T. R. & Møller, A. P. 1998. *Sperm competition and sexual selection.* New York: Academic Press.

Blaustein, A. R., Bekoff, M. & Daniels, T. J. 1987. Kin regocnition in vertebrates (excluding primates): empirical evidence. In: *Kin recognition in animals.* (Ed. by Fletcher, D. E. & Michener, C. D.), pp. 333–357. Chichester, New York: John Wiley & Sons.

Boellstorff, D. E., Owings, D. H., Penedo, M. C. T. & Hersek, M. J. 1994. Reproductive behaviour and multiple paternity of California ground squirrels. *Animal Behaviour*, **47,** 1957–1064.

Borgia, G. 1985. Bowerquality, number of decorations and mating success of male satin bowerbirds (*Ptilonorhynchus violaceus*). *Animal Behaviour*, **33,** 266–271.

Bowcock, A. M., Ruiz-Linares, A., Tomfohrde, J., Minch, E., Kidd, J. R. & Cavalli-Sforza, L. L. 1994. High resolution of human evolutionary trees with polymorphic microsatellites. *Nature*, **368,** 455–457.

Boyd, S. K. & Blaustein, A. R. 1985. Familiarity and inbreeding avoidance in the gray-tailed vole (*Microtus canicaudus*). *Journal of Mammalogy*, **66,** 348–352.

Brown, J. H., Jardetzky, T. & Saper, M. A. 1988. A hypothetical model of the foreign antigen binding site of class II histocompatibility molecules. *Nature*, **332,** 845–850.

Brown, J. L. 1987. *Helping and communal breeding in birds: ecology and evolution.* Princeton, New Jersey: Princeton University Press.

Brown, J. L. & Eklund, A. C. 1994. Kin recognition and the major histocompatibility complex: an integrative review. *The American Naturalist*, **143,** 435–461.

Brown, L. E. 1969. Field experiments on the movements of *Apodemus sylvaticus* L. using trapping and tracking techniques. *Oecologia*, **2,** 198–222.

Brown, R. E. 1986. Social and hormonal factors influencing infanticide and its suppression in adult male Long-Evans rats (*Rattus norvegicus*). *Journal of Comparative Psychology*, **100,** 155–161.

Brown, R. E. & Macdonald, D. W. 1985. *Social odours in mammals.* Oxford: Clarendon Press.

Brown, R. E., Roser, B. & Singh, P. B. 1989. Class I and class II regions of the major histocompatibility complex both contribute to individual odors in congenic inbred strains of rats. *Behavior Genetics*, **19,** 659–674.

Bruce, H. M. 1960. A block to pregnancy in the mouse caused by proximity of strange males. *Journal of Reproduction and Fertility*, **1,** 96–103.

Canova, L., Maistrello, L. & Emiliani, D. 1994. Comparative ecology of the wood mouse *Apodemus sylvaticus* in two differing habitats. *Zeitschrift für Säugetierkunde-International Journal of Mammalian Biology*, **59,** 193–198.

Chapais, B. 1988. Experimental matrilineal inheritance of rank in female Japanese macaques. *Animal Behaviour*, **36,** 1025–1037.

Charlesworth, D. & Charlesworth, B. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics*, **18,** 237–368.

Cicirello, D. M. & Wolff, J. O. 1990. The effects of mating on infanticide and pup discrimination in white-footed mice. *Behavioral Ecology and Sociobiology*, **26,** 275–279.

Clarke, F. M. & Faulkes, C. G. 1999. Kin discrimination and female mate choice in the naked mole-rat *Heterocephalus glaber*. *Proceedings of the Royal Society of London Series B - Biological Sciences*, **266,** 1995–2002.

Clutton-Brock, T. H. 1989. Mammalian mating systems. *Proceedings of the Royal Society of London Series B - Biological Sciences*, **236,** 339–372.

Clutton-Brock, T. H., Hiraiwa-Hasegawa, M. & Robertson, A. 1989. Mate choice on fallow deer leks. *Nature*, **340,** 463–465.

Collins, S. A., Gosling, L. M., Hudson, J. & Cowan, D. 1997. Does behaviour after weaning affect the dominance status of adult male mice (*Mus domesticus*)? *Behaviour*, **134,** 989–1002.

Coopersmith, C. B. & Lenington, S. 1992. Female preferences based on male quality in house mice: interaction between male dominance rank and t-complex genotype. *Ethology*, **90,** 1–16.

Crawley, M. C. 1969. Movements and home-ranges of *Clethrionomys glareolus* Schreber and *Apodemus sylvaticus* L. in north-east England. *Oikos*, **20,** 310–319.

Daly, M. & Wilson, M. 1988. Evolutionary social psychology and family homicide. *Science*, **242,** 519–524.

Darwin, C. 1871. *The descent of man, and selection in relation to sex.* London: John Murray.

Datta, S. 1983. Relative power and the acquisition of rank. In: *Primate social relationships, an integrated approach.* (Ed. by Hinde, R. A.), pp. 93–102. Oxford: Blackwell Scientific Publications.

de Waal, F. B. M. 1977. The organization of agonistic relations within two captive groups of Java monkeys (*Macaca fascicularis*). *Zeitschrift für Tierpsychologie*, **44,** 225–282.

Desjardins, C., Maruniak, J. A. & Bronson, F. H. 1973. Social rank in houce mice: differentiation revealed by ultraviolet visualization of urinary marking patterns. *Science*, **182,** 939–941.

DeVries, A. C., Johnson, C. L. & Carter, C. S. 1997. Familiarity and gender influence social preferences in prairie voles (*Microtus ochrogaster*). *Canadian Journal of Zoology*, **75,** 295–301.

Dewsbury, D. A. 1982a. Avoidance of incestuous breeding between siblings in two species of *Peromyscus* mice. *Biology of Behaviour*, **7,** 157–169.

Dewsbury, D. A. 1982b. Dominance rank, copulatory behavior and differential reproduction. *Quarterly Review of Biology*, **57,** 135–159.

Dewsbury, D. A. 1988. Kin discrimination and reproductive behavior in muroid rodents. *Behavior Genetics*, **18,** 525–536.

Dhondt, A. A. & Schillemans, J. 1983. Reproductive success of the great tit in relation to its territorial status. *Animal Behaviour*, **31,** 902–912.

Diaz, M., Gonzalez, E., Munoz-Pulido, R. & Naveso, M. A. 1993. Effects of food abundance and habitat structure on seed-eating rodents in Spain wintering in man-made habitats. *Zeitschrift für Säugetierkunde-International Journal of Mammalian Biology*, **58,** 302–311.

Díaz, M., Santos, T. & Tellería, J. L. 1999. Effects of forest fragmentation on the winter body condition and population parameters of an habitat generalist, the wood mouse *Apodemus sylvaticus*: a test of a hypotheses. *Acta Oecologica*, **20,** 39–49.

Douglass, R. J., Douglass, K. S. & Rossi, L. 1992. Ecological distribution of bank voles and wood mice in disturbed habitats: preliminary results. *Acta Theriologica*, **37,** 359–370.

Drickamer, L. C. 1992. Oestrous female house mice discriminate dominant from subordinate males and sons of dominant from sons of subordinate males by odour cues. *Animal Behaviour*, **43,** 868–870.

Drickamer, L. C., Gowaty, P. A. & Holmes, C. M. 2000. Free female mate choice in house mice affects the reproductive success and offspring viability and performance. *Animal Behaviour*, **59,** 371–378.

Dugatkin, L. A. & Godin, J.-G. J. 1992. Reversal of female mate choice by copying in the guppy (*Poecilia reticulata*). *Proceedings of the Royal Society of London Series B - Biological Sciences*, **249,** 179–184.

Egid, K. & Brown, J. L. 1989. The major histocompatibility complex and female mating preferences in mice. *Animal Behaviour*, **38,** 548–550.

Egid, K. & Lenington, S. 1985. Response of male mice to odors of females: effect of T- and H-2-locus genotype. *Behavior Genetics*, **15,** 287–295.

Eklund, A. C. 1997a. The effect of early experience on MHC-based mate preferences in two B10.W strains of mice (*Mus domesticus*). *Behavior Genetics*, **27,** 223–229.

Eklund, A. C. 1997b. The major histocompatibility complex and mating preference in wild house mice (*Mus domesticus*). *Behavioral Ecology*, **8,** 630–634.

Eklund, A. C. 1999. Use of the MHC for mate choice in wild house mice (*Mus domesticus*). *Genetica*, **104,** 245–248.

Ellegren, H., Hartman, G., Johansson, M. & Andersson, L. 1993. Major histocompatibility complex monomorphism and low levels of DNA fingerprinting variability in a reintroduced and rapidly expanding population of beavers. *Proceedings of the National Academy of Sciences of the United States of America*, **90,** 8150–8153.

Ellegren, H., Mikko, S., Wallin, K. & Anderson, L. 1996. Limited polymorphism at major histocompatibility complex (MHC) loci in the Swedish moose *A. alces*. *Molecular Ecology*, **5,** 3–9.

Elwood, R. W. 1977. Changes in the response of male and female gerbils (*Meriones unguiculatus*) towards test pups during the pregnancy of the female. *Animal Behaviour*, **25,** 46–51.

Elwood, R. W. 1985. Inhibition of infanticide and onset of paternal care in male mice (*Mus musculus*). *Journal of Comparative Psychology*, **99,** 457–468.

Elwood, R. W. & Kennedy, H. F. 1991. Selectivity in paternal and infanticidal responses by male mice: effects of relatedness, location and previous sexual partners. *Behavioral and Neural Biology*, **56,** 129–147.

Elwood, R. W. & Ostermeyer, M. C. 1984. Does copulation inhibit infanticide in male rodents? *Animal Behaviour*, **32,** 293–294.

Emlen, S. T. 1991. Evolution of cooperative breeding in birds and mammals. In: *Behavioural ecology: an evolutionary approach* (Ed. by Krebs, J. R. & Davies, N. B.), pp. 301–307. Oxford: Blackwell.

Emlen, S. T. & Oring, L. W. 1977. Ecology, sexual selection and the evolution of mating systems. *Science*, **197,** 215–223.

Fadao, T., Tingzheng, W. & Yajun, Z. 2000. Inbreeding avoidance and mate choice in the mandarin vole (*Microtus mandarinus*). *Canadian Journal of Zoology*, **78,** 2119–2125.

Fan, E., Levin, D. B., Glickman, B. W. & Logan, D. M. 1993. Limitations in the use of SSCP analysis. *Mutation Research*, **288,** 85–92.

Fernandez, F. A. S., Evans, P. R. & Dunstone, N. 1996. Population dynamics of the wood mouse *Apodemus sylvaticus* (Rodentia: Muridae) in a Sitka spruce successional mosaic. *Journal of Zoology*, **239,** 717–730.

Flowerdew, J. R. 1972. The effect of supplementary food on a population of wood mice (*Apodemus sylvaticus*). *Journal of Animal Ecology*, **41,** 553–566.

Flowerdew, J. R. 1974. Field and laboratory experiments on the social behaviour and population dynamics of the wood mouse (*Apodemus sylvaticus*). *Journal of Animal Ecology*, **43,** 499–510.

Flowerdew, J. R. 1987. *Mammals: Their reproductive biology and population ecology.* London: Edward Arnold.

Flowerdew, J. R. 2000. Wood Mice - Small Granivores/Insectivores with seasonally variable patterns. In: *Activity patterns in small mammals: an ecological approach.* (Ed. by Halle, S. & Stenseth, N. C.), pp. 177–189. Berlin; Heidelberg: Springer-Verlag.

Flowerdew, J. R. & Ellwood, S. A. 2001. Impacts of woodland deer on small mammal ecology. *Forestry*, **74,** 277–287.

Foltz, D. W. & Hoogland, J. L. 1983. Genetic evidence of outbreeding in the black-tailed prairie dog (*Cynomys ludovicianus*). *Evolution*, **37,** 273–281.

Foltz, D. W. & Schwagmeyer, P. L. 1989. Sperm competition in the thirteen-lined ground squirrel: differential fertilization succes under field conditions. *The American Naturalist*, **133,** 257–265.

Futuyma, D. J. 1997. *Evolutionary biology*. Sunderland, Massachusetts: Sinauer Associates, Inc.

Gage, M. J. G., Stockley, P. & Parker, G. A. 1995. Effects of alternative male mating strategies on characteristics of sperm production in the Atlantic salmon (*Salmo salar*): Theoretical and empirical investigations. *Philosophical Transactions of the Royal Society of London Series B - Biological Science*, **350,** 391–399.

Galef, B. G., Jr. & White, D. J. 1998. Mate-choice copying in Japanese quail, *Coturnix coturnix japonica*. *Animal Behaviour*, **55,** 545–552.

Gaston, A. J. 1978. The evolution of group territorial behavior and cooperative breeding. *The American Naturalist*, **112,** 1091–1100.

Gavish, L., Hofmann, J. E. & Getz, L. L. 1984. Sibling recognition in the prairie vole, *Microtus ochrogaster*. *Animal Behaviour*, **32,** 362–366.

Gerlach, G. 1990. Dispersal mechanisms in a captive wild house mouse population (*Mus domesticus* Rutty). *Biological Journal of the Linnean Society*, **47,** 271–277.

Gerlach, G. 1996. Emigration mechanisms in feral house mice - a laboratory investigation of the influence of social structure, population density and aggression. *Behavioral Ecology and Sociobiology*, **39,** 159–170.

Gerlach, G. 1998. Impact of social ties on dispersal, reproduction and dominance in feral house mice (*Mus musculus domesticus*). *Ethology*, **104,** 487–499.

Gerlach, G. & Bartmann, S. 2002. Reproductive skew, costs and benefits of cooperative breeding in female wood mice (*Apodemus sylvaticus*). *Behavioral Ecology and Sociobiology*, **52**.

Gibson, R. M., Bradbury, J. W. & Vehrencamp, S. L. 1991. Mate choice in lekking sage grouse revisited the roles of vocal display female site fidelity and copying. *Behavioral Ecology*, **2,** 165–180.

Gibson, R. M. & Höglund, J. 1992. Copying and sexual selection. *Trends in Ecology and Evolution*, **7,** 229–231.

Gilder, P. M. & Slater, P. J. B. 1978. Interest of mice in conspecific male odours is influenced by degree of kinship. *Nature*, **274,** 364–365.

Ginsberg, J. R. & Huck, U. W. 1989. Sperm competition in mammals. *Trends in Ecology and Evolution*, **4,** 74–79.

Gockel, J., Harr, B., Schlötterer, C., Arnold, W., Gerlach, G. & Tautz, D. 1997. Isolation and characterisation of microsatellite loci from *Apodemus flavicollis* (Rodentia, Muridae) and *Clethrionomys glareolus* (Rodentia, Cricetidae). *Molecular Ecology*, **6,** 597–599.

Goldizen, A. W., Putland, D. A. & Goldizen, A. R. 1998. Variable mating patterns in Tasmanian native hens (*Gallinula mortierii*): Correlates of reproductive success. *Journal of Animal Ecology*, **67,** 307–317.

Gorman, M. L. & Zubaid, A. b. M. A. 1993. A comparative study of the ecology of woodmice *Apodemus sylvaticus* in two contrasting habitats: deciduous woodland and maritime sand-dunes. *Journal of Zoology, London*, **229,** 385–396.

Gosling, L. M. & McKay, H. V. 1990. Competitor assessment by scent matching: an experimental test. *Behavioral Ecology and Sociobiology*, **26,** 415–420.

Gosling, L. M., Roberts, S. C., Thornton, E. A. & Andrew, M. J. 2000. Life history costs of olfactory status signalling in mice. *Behavioral Ecology and Sociobiology*, **48,** 328–332.

Goudet, J. 1995. Fstat version 1.2: a computer program to calculate Fstatistics. *Journal of Heredity*, **86,** 485–486.

Grant, J. W. A. & Green, L. D. 1996. Mate copying versus preference for actively courting males by female Japanese medaka (*Oryzias latipes*). *Behavioral Ecology*, **7,** 165–167.

Grau, H. J. 1982. Kin recognition in white-footed deermice (*Peromyscus leucopus*). *Animal Behaviour*, **30,** 497–505.

Greenwood, P. J. 1980. Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour*, **28,** 1140–1162.

Grob, B., Knapp, L. A., Martin, R. D. & Anzenberger, G. 1998. The major histocompatibiliy complex and mate choice: inbreeding avoidance and selection of good genes. *Experimental and Clinical Immunogenetics*, **15,** 119–129.

Günther, E. 1996. Current status of the molecular genetic analysis of the rat major histocompatibility complex. *Folia Biologica (Praha)*, **42,** 129–145.

Gwynne, D. T. 1984. Courtship feeding increases female reproductive success in bushcrickets. *Nature*, **307,** 361–363.

Halle, S. 1988. Avian predation upon a mixed community of common voles (*Microtus arvalis*) and wood mice (*Apodemus sylvaticus*). *Oecologia*, **75,** 451–455.

Halle, S. 1993. Wood mice (*Apodemus sylvaticus* L.) as pioneers of recolonization in an reclaimed area. *Oecologia*, **94,** 120–127.

Halpin, Z. T. 1991. Kin recognition cues in vertebrates. In: *Kin recognition* (Ed. by Hepper, P. G.), pp. 220–258. Cambridge: Cambridge University Press.

Halpin, Z. T. & Hoffman, M. D. 1987. Sibling recognition in the white-footed mouse, *Peromyscus leucopus*: association or phenotype matching? *Animal Behaviour*, **35,** 563–570.

Hamilton, W. D. 1964. The genetical evolution of social behaviour. I and II. *Journal of Theoretical Biology*, **7,** 1–52.

Hanken, J. & Sherman, P. W. 1981. Multiple paternity in Belding's ground squirrel litters. *Science*, **212,** 351–353.

Harr, B., Musolf, K. & Gerlach, G. 2000. Characterization and isolation of DNA microsatellite primers in wood mice (*Apodemus sylvaticus*, Rodentia). *Molecular Ecology*, **9,** 1664–1665.

Harris, M. A. & Murie, J. O. 1984. Inheritance of nest sites in female Columbian ground squirrel. *Behavioral Ecology and Sociobiology*, **15,** 97–102.

Hartl, D. L. & Clark, A. G. 1997. *Principles of Population Genetics.* Sunderland, Massachusetts: Sinauer Associates, Inc.

Hasselquist, D., Bensch, S. & von Schantz, T. 1996. Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. *Nature*, **381,** 229–232.

Hausfater, G. 1984. Infanticide in langurs: strategies, counterstrategies, and parameter values. In: *Infanticide: comparative and evolutionary perspectives* (Ed. by Hausfater, G. & Hrdy, S. B.), pp. 257–282. New York: Aldine Publishing Company.

Hausfater, G. & Hrdy, S. B. 1984. *Infanticide: comparative and evolutionary perspectives.* New York: Aldine Publishing Company.

Hayashi, K. 1992. PCR-SSCP: A method for detection of mutations. *Genetic Analysis, Techniques & Applications*, **9,** 73–79.

Hedrick, P. W. & Black, F. L. 1997. HLA and mate selection: no evidence in South Amerindians. *American Journal of Human Genetics*, **61,** 505–511.

Hedrick, P. W. & Löschcke, V. 1996. MHC and mate selection in humans? *Trends in Ecology & Evolution*, **11,** 24.

Hepper, P. G. 1983. Sibling recognition in the rat. *Animal Behaviour*, **31,** 1177–1191.

Heth, G., Todrank, J. & Johnston, R. E. 1998. Kin recognition in golden hamsters: Evidence for phenotypic matching. *Animal Behaviour*, **56,** 409–417.

Hill, G. E. 1990. Female house finches prefer colourful males: sexual selection for a condition-dependent trait. *Animal Behaviour*, **40,** 563–572.

Hill, J. L. 1974. Peromyscus: effect of early pairing on reproduction. *Science*, **186,** 1042–1044.

Hoffmeyer, I. 1982. Responses of female bank voles (*Clethrionomys glareolus*) to dominant vs subordinate conspecific males and to urine odors from dominant vs subordinate males. *Behavioral and Neural Biology*, **36,** 178–188.

Höglund, J., Alatalo, R. V., Gibson, R. M. & Lundberg, A. 1995. Mate-choice copying in black grouse. *Animal Behaviour*, **49,** 1627–1633.

Holmes, W. G. 1984. Sibling recognition in thirteen-lined ground squirrels: effects of relatedness, rearing association, and olfaction. *Behavioral Ecology and Sociobiology*, **14,** 225–233.

Holmes, W. G. & Sherman, P. W. 1982. The ontogeny of kin recognition in two species of ground squirrels. *American Zoologist*, **22,** 491–517.

Holmes, W. G. & Sherman, P. W. 1983. Kin recognition in animals. *American Scientist*, **71,** 46–54.

Hongyo, T., Buzard, G. S., Calvert, R. J. & Weghorst, C. M. 1993. "Cold SSCP": A simple, rapid and non-radioactive method for optimized single-strand conformation polymorphism analyses. *Nucleic Acids Research*, **21,** 3637–3642.

Hoogland, J. L. 1981. The evolution of coloniality in white-tailed and black-tailed prairie dogs (Sciuridae: *Cynomys leucurus* and *C. ludovicianus*). *Ecology*, **62,** 252–272.

Hoogland, J. L. 1982. Prairie dogs avoid inbreeding. *Science*, **215,** 1639–1641.

Hoogland, J. L. 1998. Why do Gunnison's prairie dogs copulate with more than one male? *Animal Behaviour*, **55,** 351–359.

Hoogland, J. L. & Sherman, P. W. 1976. Advantages and disadvantages of bank swallow (*Riparia riparia*) coloniality. *Ecological Monographs*, **46,** 33–58.

Horne, T. J. & Ylönen, H. 1996. Female bank voles (*Clethrionomys glareolus*) prefer dominant males; but what if there is no choice? *Behavioral Ecology and Sociobiology*, **38,** 401–405.

Horne, T. J. & Ylönen, H. 1998. Heritabilities of dominance-related traits in male bank voles (*Clethrionomys glareolus*). *Evolution*, **52,** 894–899.

Horrocks, J. & Hunte, W. 1983. Maternal rank and offspring rank in vervet monkeys: an appraisal of the mechanisms of rank acquisition. *Animal Behaviour*, **31,** 772–782.

Hosken, D. J. 1998. Sperm fertility and skewed paternity during sperm competition in the Australian long-eared bat, *Nyctophilus geoffroyi* (Chiroptera, Verspertilionidae). *Journal of Zoology*, **245,** 93–100.

Hosken, D. J. & Blanckenhorn, W. U. 1999. Female multiple mating, inbreeding avoidance and fitness: it´s not only the magnitude of costs and benefits that count. *Behavioral Ecology*, **10,** 462–464.

Howard, R. D. 1980. Mating behaviour and mating success in bullfrogs, *Rana catesbiana*. *Animal Behaviour*, **28,** 705–716.

Hrdy, S. B. 1979. Infanticide among animals: a review, classification, and examination of the implications for the reproductive strategies of females. *Ethology and Sociobiology*, **1,** 13–40.

Hrdy, S. B. 2000. The optimal number of fathers. Evolution, demography, and history in the shaping of female mate preferences. *Annals New York Academy of Sciences*, **907,** 75–96.

Huck, U. W. & Banks, E. M. 1982. Differential attraction of females to dominant males: olfactory discrimination and mating preferences in the brown lemming (*Lemmus trimucronatus*). *Behavioral Ecology and Sociobiology*, **11,** 217–222.

Huck, U. W., Banks, E. M. & Wang, S.-C. 1981. Olfactory discrimination of social status in the brown lemming. *Behavioral and Neural Biology*, **33,** 364–371.

Huck, U. W., Soltis, R. L. & Coopersmith, C. B. 1982. Infanticide in male laboratory mice: effects of social status, prior sexual experience, and basis for social discrimination between related and unrelated young. *Animal Behaviour*, **30,** 1158–111.

Hughes, A. L. & Yeager, M. 1998. Natural selection at major histocompatibility complex loci of vertebrates. *Annual Review of Genetics*, **32,** 415–435.

Hughes, C. R. & Queller, D. C. 1993. Detection of highly polymorphic microsatellite loci in a species with little allozyme polymorphism. *Molecular Ecology*, **2,** 131–137.

Hugo, A. 1990. Wiederfangerfolg bei Kleinsäugern mit einer neuen Markierungsmethode. *Zeitschrift für Säugetierkunde-International Journal of Mammalian Biology*, **55,** 421–424.

Hurst, J. L. 1987. Behavioural variation in wild house mice (*Mus domesticus* Rutty): a quantitative assessment of female social organization. *Animal Behaviour*, **35,** 1846–1857.

Hurst, J. L. 1990. Urine marking in populations of wild house mice, *Mus domesticus* Rutty. III. Communication between the sexes. *Animal Behaviour*, **40,** 233–243.

Hurst, J. L. & Nevison, C. M. 1994. Do female house mice, *Mus domesticus*, regulate their exposure to reproductive priming pheromones? *Animal Behaviour*, **48,** 945–959.

Hurst, J. L., Payne, C. E., Nevison, C. M., Marie, A. D., Humphries, R. E., Robertson, D. H. L., Cavaggioni, A. & Beynon, R. J. 2001. Individual recognition in mice mediated by major urinary proteins. *Nature*, **414,** 631–634.

Hurst, J. L., Robertson, D. H. L., Tolladay, U. & Beynon, R. J. 1998. Proteins in urine scent marks of male house mice extend the longevity of olfactory signals. *Animal Behaviour*, **55,** 1289–1297.

Ishibashi, Y., Saitoh, T., Abe, S. & Yoshida, M. C. 1997. Sex-related spatial kin structure in a spring population of grey-sided voles *Clethrionomys rufocanus* as revealed by mitochondrial and microsatellite DNA analyses. *Molecular Ecology*, **6,** 63–71.

Jamieson, I. G. & Colgan, P. W. 1989. Eggs in the nests of males and their effect on mate choice in the three-spined stickleback. *Animal Behaviour*, **38,** 859–865.

Jennions, M. D. & Petrie, M. 1997. Variation in mate choice and mating preferences: a review of causes and consequences. *Biological Reviews of the Cambridge Philosophical Society*, **72,** 283–327.

Jennions, M. D. & Petrie, M. 2000. Why do females mate multiply? A review of the genetic benefit. *Biological Reviews of the Cambridge Philosophical Society*, **75,** 21–64.

Jiménez, J. A., Hughes, K. A., Alaks, G., Graham, L. & Lacy, R. C. 1994. An experimental study of inbreeding depression in a natural habitat. *Science*, **266,** 271–273.

Jin, K., Ho, H.-N., Speed, T. P. & Gill, T. J. I. 1995. Reproductive failure and the major histocompatibility complex. *American Journal of Human Genetics*, **56,** 1456–1467.

Jonsson, P. & Silverin, B. 1997. The estrous cycle in female wood mice (*Apodemus sylvaticus*) and the influence of male. *Annales Zoologici Fennici*, **34,** 197–204.

Jordan, W. C. & Bruford, M. W. 1998. New perspectives on mate choice and the MHC. *Heredity*, **81,** 127–133.

Kareem, A. M. & Barnard, C. J. 1982. The importance of kinship and familiarity in social interactions between mice. *Animal Behaviour*, **30,** 594–601.

Kavaliers, M., Colwell, D. D., Ossenkopp, K.-P. & Perrot-Sinal, T. S. 1997. Altered responses to female odors in parasitized male mice: neuromodulatory mechanisms and relations to female choice. *Behavioral Ecology and Sociobiology*, **40,** 373–384.

Kavaliers, M., Wiebe, J. P. & Galea, L. A. M. 1994. Male preference for the odors of estrous female mice is enhanced by the neurosteroid 3α-hydroxy-4-pregnen-20-one (3αHP). *Brain Research*, **646,** 140–144.

Kawata, M. 1990. Fluctuating populations and kin interaction in mammals. *Trends in Ecology and Evolution*, **5,** 17–20.

Keane, B. 1990. The effect of relatedness on reproductive success and mate choice in the white-footed mouse, *Peromyscus leucopus*. *Animal Behaviour*, **39,** 264–273.

Kirkpatrick, M. & Ryan, M. J. 1991. The evolution of mating preferences and the paradox of lek. *Nature*, **350,** 33–38.

Kleiman, D. G. 1977. Monogamy in Mammals. *The Quarterly Review of Biology*, **52,** 39–66.

Knapp, L. A., Ha, J. C. & Sackett, G. P. 1996. Parental MHC antigen sharing and pregnancy wastage in captive pigtailed macaques. *Journal of Reproduction and Immunology*, **32,** 73–88.

Koenig, W. D. & Mumme, R. L. 1987. *Population ecology of the cooperatively breeding acorn wood pecker.* Princeton, NJ: Princeton University Press.

König, B. 1993. Maternal investment of communally nursing female house mice (*Mus musculus domesticus*). *Behavioural Processes*, **30,** 61–74.

König, B. 1994. Components of lifetime reproductive success in communally and solitarily nursing house mice - a laboratory study. *Behavioral Ecology and Sociobiology*, **34,** 275–283.

König, B. & Markl, H. 1987. Maternal care in house mice. *Behavioral Ecology and Sociobiology*, **20,** 1–9.

Krackow, S. & Matuschak, B. 1991. Mate choice for non-siblings in wild house mice: evidence from a choice test and a reproductive test. *Ethology*, **88,** 99–108.

Krebs, J. R. 1971. Territory and breeding density in the great tit, *Parus major* L.. *Ecology*, **52,** 2–22.

Lacy, R. C., Alaks, G. A. & Walsh, A. 1996. Hierarchical analysis of inbreeding depression in *Peromyscus polionotus*. *Evolution*, **50,** 2187–2200.

Lambin, X. & Krebs, C. J. 1991a. Can changes in female relatedness influence microtine population dynamics? *Oikos*, **91,** 126–132.

Lambin, X. & Krebs, C. J. 1991b. Spatial organization and mating system of *Microtus townsendii*. *Behavioral Ecology and Sociobiology*, **28,** 353–363.

Lambin, X. & Krebs, C. J. 1993. Influence of female relatedness on the demography of Townsend´s vole populations in spring. *Journal of Animal Ecology*, **62,** 536–550.

Law, J. C., Facher, E. A. & Deka, A. 1996. Nonradioactive single-strand conformation polymorphism analysis with application for mutation detection in a mixed population of cells. *Analytical Biochemistry*, **236,** 373–375.

Lenington, S. & Egid, K. 1985. Female discrimination of male odors correlated with male genotype at the T locus: a response to T-locus of H-2-locus variability? *Behavior Genetics*, **15,** 53–67.

Lessa, E. P. & Applebaum, G. 1993. Screening techniques for detecting allelic variation in DNA sequences. *Molecular Ecology*, **2,** 119–129.

Madsen, T., Shine, R., Loman, J. & Håkansson, T. 1992. Why do female adders copulate so frequently? *Nature*, **355,** 440–441.

Manning, C. J., Dewsbury, D. A., Wakeland, E. K. & Potts, W. K. 1995. Communal nesting and communal nursing in house mice, *Mus musculus domesticus*. *Animal Behaviour*, **50,** 741–751.

Manning, C. J., Wakeland, E. K. & Potts, W. K. 1992. Communal nesting patterns in mice implicate MHC genes in kin recognition. *Nature*, **360,** 581–583.

Mappes, T., Ylönen, H. & Viitala, J. 1995. Higher reproductive success among kin groups of bank voles (*Clethrionomys glareolus*). *Ecology*, **76,** 1276–1282.

Margulis, S. W. 1998. Differential effects of inbreeding at juvenile and adult life-history stages in *Peromyscus polionotus*. *Journal of Mammalogy*, **79,** 326–336.

Marshall, T. C., Slate, J., Kruuk, L. E. B. & Pemberton, J. M. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7,** 639–655.

Martin, Y., Gerlach, G., Schlötterer, C. & Meyer, A. 2000. Molecular phylogeny of European muroid rodents based on complete cytochrom *b* sequences. *Molecular Phylogenetics and Evolution*, **15,** 37–47.

Mateo, J. M. & Johnston, R. E. 2000. Kin recognition and the 'armpit effect': evidence of self-referent phenotype matching. *Proceedings of the Royal Society of London Series B - Biological Sciences*, **267,** 695–700.

McClintock, M. K., Anisko, J. J. & Adler, N. T. 1982. Group mating among norway rats II. The social dynamics of copulation: competition, cooperation, and mate choice. *Animal Behaviour*, **30,** 410–425.

McCracken, G. F. & Bradbury, J. W. 1977. Paternity and genetic heterogeneity in the polygynous bat *Phyllostomus hastatus*. *Science*, **198,** 303–306.

McGuire, K. L., Duncan, W. R. & Tucker, P. W. 1985. Syrian hamster DNA shows limited polymorphism at class I-like loci. *Immunogenetics*, **22,** 257–268.

McShea, W. J. & Madison, D. M. 1984. Communal nesting between reproductive active females in a spring population of *Microtus pennsylvanicus*. *Canadian Journal of Zoology*, **62,** 344–346.

Meagher, S., Penn, D. J. & Potts, W. K. 2000. Male-male competition magnifies inbreeding depression in wild house mice. *Proceedings of the National Academy of Sciences of the United States of America*, **97,** 3324–3329.

Meagher, S. & Potts, W. K. 1997. A microsatellite-based MHC genotyping system for house mice (*Mus domesticus*). *Hereditas*, **127,** 75–82.

Milinski, M. & Bakker, T. C. M. 1990. Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. *Nature*, **344,** 330–333.

Møller, A. P. 1990. Effects of a haematophagous mite on the barn swallow (*Hirundo rustica*): a test of the Hamilton and Zuk hypothesis. *Evolution*, **44,** 771–784.

Møller, A. P. & Alatalo, R. V. 1999. Good-genes effects in sexual selection. *Proceedings of the Royal Society of London Series B - Biological Sciences*, **266,** 85–91.

Montgomery, W. I. & Gurnell, J. 1985. The behaviour of *Apodemus*. *Symposia of the Zoological Society of London*, **55,** 89–115.

Montgomery, W. I., Wilson, W. L. & Elwood, R. W. 1997. Spatial regulation and population growth in the wood mouse *Apodemus sylvaticus*: experimental manipulations of males and females in natural populations. *Journal of Animal Ecology*, **66,** 755–768.

Montgomery, W. I., Wilson, W. L., Hamilton, R. & McCartney, P. 1991. Dispersion in the wood mouse, *Apodemus sylvaticus*: variable resources in time and space. *Journal of Animal Ecology*, **60,** 179–192.

Murie, J. O. 1995. Mating behavior of Columbian ground squirrels. I. Multiple mating by females and multiple paternity. *Canadian Journal of Zoology*, **73,** 1819–1826.

Musolf, K. 1996. Isolationswirkung von Verkehrswegen auf Kleinsäugerpopulationen - populationsgenetische und verhaltensökologische Untersuchung. In: *Diplomarbeit der Fakultät für Biologie*. Universität Konstanz: Germany.

Nelson, J. 1994. Determinants of spacing behaviour, reproductive success and mating system in male field voles, *Microtus agrestis*. *PhD Thesis, Department of Animal Ecology, Lund University, Sweden*.

Niethammer, J. & Krapp, F. 1982. *Handbuch der Säugetiere Europas. Band 2/I. Nagetiere II.* Wiesbaden: Akademische Verlagsgesellschaft.

Noonan, K. C. 1983. Female choice in the cichlid fish *Cichlasoma nigrofasciatum*. *Animal Behaviour*, **31,** 1005–1010.

Norris, K. 1993. Heritable variation in a plumage indicator of viability in male great tits *Parus major*. *Nature*, **362,** 537–539.

Novotny, M., Harvey, S. & Jemiolo, B. 1990. Chemistry of male dominance in the house mouse, *Mus domesticus*. *Experientia*, **46,** 109–113.

Ober, C. 1995. HLA and reproduction: Lessons from studies in the Hutterites. *Placenta*, **16,** 569–577.

Ober, C., Weitkamp, L. R., Cox, N., Dytch, H., Kostyu, D. & Elias, S. 1997. HLA and mate choice in humans. *American Journal of Human Genetics*, **61,** 497–504.

O'Connell, S. M. & Cowlishaw, G. 1994. Infanticide avoidance, sperm competition and mate choice: The function of copulation calls in female baboons. *Animal Behaviour*, **48,** 687–694.

Olsén, K. H., Grahn, M., Lohm, J. & Langefors, Å. 1998. MHC and kin discrimination in juvenile Arctic charr, *Salvelinus alpinus* (L.). *Animal Behaviour*, **56,** 319–327.

Olsson, M., Gullberg, A., Tegelström, H., Madsen, T. & Shine, R. 1994. Can female adders multiply? *Nature*, **369,** 528.

Orita, M., Suzuki, Y., Sekiya, T. & Hayashi, K. 1989. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics*, **5,** 874–879.

Packer, C. & Pusey, A. E. 1983. Adaptations of female lions to infanticide by incoming males. *The American Naturalist*, **121,** 716–723.

Pearse, D. E., Janzen, F. J. & Avise, J. C. 2002. Multiple paternity, sperm storage, and reproductive success of female and male painted turtles (*Chrysemys picta*) in nature. *Behavioral Ecology and Sociobiology*, **51,** 164–171.

Penn, D. J. & Potts, W. K. 1998. MHC-disassortative mating preferences reversed by cross-fostering. *Proceedings of the Royal Society London Series B - Biological Sciences*, **265,** 1299–1306.

Penn, D. J. & Potts, W. K. 1999. The evolution of mating preferences and major histocompatibility complex genes. *The American Naturalist*, **153,** 145–164.

Perrigo, G., Bryant, W. C. & vom Saal, F. S. 1990. A unique timing system prevents male mice from harming their own offspring. *Animal Behaviour*, **39,** 535–539.

Petrie, M. 1994. Improved growth and survival of offspring of peacocks with more elaborate trains. *Nature*, **371,** 598–599.

Pillay, N. 1998. The effects of inbreeding in two otomyine rodents. *Mammalia*, **62,** 521–528.

Pillay, N. 2000. Female mate preference and reproductive isolation in populations of the striped mouse *Rhabdomys pumilio*. *Behaviour*, **137,** 1431–1441.

Porter, R. H., Matochik, J. A. & Makin, J. W. 1983. Evidence for phenotype matching in spiny mice (*Acomys cahirinus*). *Animal Behaviour*, **31,** 978–984.

Porter, R. H., Wyrick, M. & Pankey, J. 1978. Sibling recognition in spiny mice (*Acomys cahirinus*). *Behavioral Ecology and Sociobiology*, **3,** 61–68.

Potts, W. K., Manning, C. J. & Wakeland, E. K. 1991. Mating patterns in seminatural populations of mice influenced by MHC genotype. *Nature*, **352,** 619–621.

Potts, W. K., Manning, C. J. & Wakeland, E. K. 1994. The role of infectious disease, inbreeding and mating preferences in maintaining MHC genetic diversity: an experimental test. *Philosophical Transactions of the Royal Society of London Series B - Biological Science*, **346,** 369–378.

Potts, W. K. & Slev, P. R. 1995. Pathogen-based models favoring MHC-genetic diversity. *Immunological Review*, **143,** 181–197.

Potts, W. K. & Wakeland, E. K. 1993. Evolution of MHC genetic diversity: a tale of incest, pestilence and sexual preference. *Trends in Genetics*, **9,** 408–412.

Pruett-Jones, S. 1992. Independent versus nonindependent mate choice: do females copy each other? *The American Naturalist*, **140,** 1000–1009.

Pugh, S. R. & Tamarin, R. H. 1991. A comparison of population characteristics and reproductive success of resident and immigrant meadow voles. *Canadian Journal of Zoology*, **69,** 2638–2643.

Pusenius, J., Viitala, J., Marienberg, T. & Ritvanen, S. 1998. Matrilineal kin clusters and their effect on reproductive success in the field vole *Microtus agrestis*. *Behavioral Ecology*, **9,** 85–92.

Pusey, A. E. & Packer, C. 1994. Infanticide in lions: consequences and counter-strategies. In: *Infanticide and parental care.* (Ed. by Parmigiani, S. & vom Saal, F. S.), pp. 277–299. London: Harwood.

Putland, D. A. & Goldizen, A. W. 2001. Family dynasties in the Tasmanian native hen (*Gallinula mortierii*). *Behavioral Ecology and Sociobiology*, **51,** 26–32.

Queller, D. C., Zacchi, F., Cervo, R., Turillazzi, S., Henshaw, M. T., Santorelli, L. A. & Strassmann, J. E. 2000. Unrelated helpers in a social insect. *Nature*, **405,** 784–787.

Qvarnström, A. & Forsgren, E. 1998. Should females prefer dominant males? *Trends in Ecology and Evolution*, **13,** 498–501.

Ralls, K. & Ballou, J. D. 1983. Extinction: lessons from zoos. In: *Genetics and conservation: a reference for managing wild animal and plant populations.* (Ed. by Schonewald-Cox, C. M., Chambers, S. M., MacBryde, B. & Thomas, L.), pp. 164–184. London: The Benjamin/Cummings Publishing Company, Inc.

Ralls, K., Harvey, P. H. & Lyles, A. M. 1986. Inbreeding in natural populations of birds and mammals. In: *Conservation Biology. The science of scarity and diversity.* (Ed. by Soulé, M. E.), pp. 35–56. Sunderland, Massachusetts: Sinauer Associates, Inc.

Rassmann, K., Schlötterer, C. & Tautz, D. 1991. Isolation of simple-sequence loci for use in polymerase chain reaction-based DNA-fingerprinting. *Electrophoresis*, **12,** 113–118.

Ratkiewicz, M. & Borkowska, A. 2000. Multiple paternity in the bank vole (*Clethrionomys glareolus*): field and experimental data. *Zeitschrift für Säugetierkunde-International Journal of Mammalian Biology*, **65,** 6–14.

Raymond, M. & Rousset, F. 1995. GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86,** 248–249.

Reich, L. M., Wood, K. M., Rothstein, B. E. & Tamarin, R. H. 1982. Aggressive behavior of male *Microtus breweri* and its demographic implications. *Animal Behaviour*, **30,** 117–122.

Reusch, T. B. H., Häberli, M. A., Aeschlimann, P. B. & Milinski, M. 2001. Female sticklebacks counts alleles in a strategy of sexual selection explaining MHC polymorphism. *Nature*, **414,** 300–302.

Reyer, H.-U. 1980. Flexible helper structure as an ecological adaptation in the pied kingfisher (*Ceryle rudis*). *Behavioral Ecology and Sociobiology*, **6,** 219–227.

Rich, T. J. & Hurst, J. L. 1998. Scent marks as reliable signals of the competitive ability of mates. *Animal Behaviour*, **56,** 727–735.

Robitaille, J. A. & Bovet, J. 1976. Field observations on the social behaviours of the Norway rat *Rattus norvegicus* (Barkenhout). *Biology of Behaviour*, **1,** 289–308.

Rosakis, A. 1999. Populationsgenetische Untersuchungen an Gelbhalsmäusen (*Apodemus flavicollis*). In: *Diplomarbeit der Fakultät für Biologie*. Universität Konstanz: Germany.

Rülicke, T., Chapuisat, M., Homberger, F. R., Macas, E. & Wedekind, C. 1998. MHC-genotype of progeny influenced by parental infection. *Proceedings of the Royal Society of London Series B - Biological Sciences*, **265,** 711–716.

Rutberg, A. T. 1983. The evolution of monogamy in primates. *Journal of Theoretical Biology*, **104,** 83–112.

Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, **239,** 487–491.

Salo, A. L. & Dewsbury, D. A. 1995. Three experiments on mate choice in meadow voles (*Microtus pennsylvanicus*). *Journal of Comparative Psychology*, **109,** 42–46.

Sambrook, J., Fritsch, E. F. & Maniatis, T. 1989. *Molecular cloning: a laboratory manual.* Cold Spring Harbor, N. Y.: Cold Spring Harbor Laboratory Press.

SAS Institute Inc. 2001. *JMP statistics and graphics guide (Version 4.0.5).*: Cary, NC.

Schlötterer, C. 1993. Non-radioactive analysis of multiplexed microsatellite reactions using a direct blotting-sequencing apparatus. *Nucleic Acids Research*, **20,** 780.

Schlupp, I. & Ryan, M. J. 1996. Mixed-species shoals and the maintenance of a sexual-asexual mating system in mollies. *Animal Behaviour*, **52,** 885–890.

Schulte, M. C. 1997. Populationsstruktur und -dynamik, Aggression und genetische Zusammensetzung einer freilebenden Waldmauspopulation (*Apodemus sylvaticus*). In: *Dissertation der Fakultät für Biologie*. Universität Konstanz: Germany.

Schwagmeyer, P. L. & Parker, G. A. 1987. Queuing for mates in the thirteen lined ground squirrels. *Animal Behaviour*, **35,** 1015–1025.

Schwartz, O. A. & Armitage, K. B. 1980. Genetic variation in social mammals: The marmot model. *Science*, **207,** 665–667.

Seddon, J. M. & Baverstock, P. R. 1998. Eight rat *RT1.Ba* sequences. *Immunogenetics*, **48,** 161–162.

Seddon, J. M. & Baverstock, P. R. 1999. Variation on islands: major histocompatibility complex (*Mhc*) polymorphism in populations of the Australian bush rat. *Molecular Ecology*, **8,** 2071–2079.

Shapiro, L. E. & Dewsbury, D. A. 1986. Male dominance, female choice and male copulatory behavior in two species of voles (*Microtus ochrogaster* and *Microtus montanus*). *Behavioral Ecology and Sociobiology*, **18,** 267–274.

Sharpe, S. T. & Millar, J. S. 1991. Influence on the variation in initiation of breeding in *Peromyscus maniculatus*. *Canadian Journal of Zoology*, **69,** 698–705.

Sheldon, B. C. 1994. Male phenotype, fertility, and the pursuit of extra-pair copulations by female birds. *Proceedings of the Royal Society of London Series B - Biological Sciences*, **257,** 25–30.

Shepher, J. 1971. Mate selection among second generation Kibbutz adolescents and adults: Incest avoidance and negative imprinting. *Archives of Sexual Behaviour*, **1,** 293–307.

Sheridan, M. & Tamarin, R. H. 1986. Kinships in a natural meadow vole population. *Behavioral Ecology and Sociobiology*, **19,** 207–211.

Sherman, P. W. 1991. Multiple mating and kin recognition by self-inspection. *Ethology and Sociobiology*, **12,** 377–386.

Sherman, P. W., Reeve, H. K. & Pfennig, D. W. 1997. Recognition systems. In: *Behavioural ecology: an evolutionary approach.* (Ed. by Krebs, J. R. & Davies, N. B.), pp. 69–96. Oxford UK: Blackwell Science.

Siegel, S. 1997. *Nichtparametrische statistische Methoden.* Eschborn: Verlag Dietmar Klotz GmbH.

Simmons, L. W., Stockley, P., Jackson, R. L. & Parker, G. A. 1996. Sperm competition or sperm selection: No evidence for female influence over paternity in yellow dung flies *Scatophaga stercoraria*. *Behavioral Ecology & Sociobiology*, **38,** 199–206.

Singer, A. G., Beauchamp, G. K. & Yamazaki, K. 1997. Volatile signals of the major histocompatibility complex in male mouse urine. *Proceedings of the National Academy of Sciences of the United States of America*, **94,** 2210–2214.

Singh, P. B. 2001. Chemosensation and genetic individuality. *Reproduction*, **121,** 529–539.

Slade, R. W. 1992. Limited MHC polymorphism in the southern elephant seal: implications for MHC evolution and marine mammal population biology. *Proceedings of the Royal Society of London Series B - Biological Sciences*, **249,** 163–171.

Slade, R. W., Moritz, C., Heideman, A. & Hale, P. T. 1993. Rapid assessment of single-copy nuclear DNA variation in diverse species. *Molecular Ecology*, **2,** 359–373.

Slate, J., Marshall, T. C. & Pemberton, J. M. 2000. A retrospective assessment of the accuracy of the paternity inference program CERVUS. *Molecular Ecology*, **9,** 801–808.

Snell, G. D. 1941. *Reproduction. Biology of the laboratory mouse.* New York: Dover Publications.

Solomon, N. G. & French, J. A. 1997. *Cooperative breeding in mammals.* Cambridge: Cambridge University Press.

Sommer, S. & Tichy, H. 1999. Major histocompatibility complex (MHC) class II polymorphism and paternity in the monogamous *Hypogeomys antimena*, the endangered, largest endemic Malagasy rodent. *Molecular Ecology*, **8,** 1259–1272.

Soroker, V. & Terkel, J. 1988. Changes in incidence of infanticidal and parental responses during the reproductive cycle in male and female wild mice *Mus musculus*. *Animal Behaviour*, **36,** 1275–1281.

Stacey, P. B. & Koenig, W. D. 1990. *Cooperative breeding in birds: long terms studies of ecology and behavior.* Cambridge: Cambridge University Press.

Stockley, P., Searle, J. B., Macdonald, D. W. & Jones, C. S. 1993. Female multiple mating behaviour in the common shrew as a strategy to reduce inbreeding. *Proceedings of the Royal Society of London Series B - Biological Sciences*, **254,** 173–179.

Stopka, P. & Macdonald, D. W. 1998. Signal interchange during mating in the wood mouse (*Apodemus sylvaticus*): the concept of active and passive signalling. *Behaviour*, **135,** 231–249.

Summers, K. & Amos, W. 1997. Behavioral, ecological, and molecular genetic analyses of reproductive strategies in the Amazonian dart-poison frog, *Dendrobates ventrimaculatus*. *Behavioral Ecology*, **8,** 260–267.

Taborsky, M. 1984. Broodcare helpers in the cichlid fish *Lamprologus brichardi*: their costs and benefits. *Animal Behaviour*, **32,** 1236–1252.

Taborsky, M. 1985. Breeder-helper conflict in a cichlid fish with brood-care helpers: an experimental analysis. *Behaviour*, **95,** 45–75.

Takahata, N., Satta, Y. & Klein, J. 1992. Polymorphism and balancing selection at major histocompatibility complex loci. *Genetics*, **130,** 925–938.

Tang-Martinez, Z. 2001. The mechanisms of kin discrimination and the evolution of kin recognition in vertebrates: a critical re-evaluation. *Behavioural Processes*, **53,** 21–40.

Tew, T. E. & Macdonald, D. W. 1994. Dynamics of space use and male vigour amongst wood mice, *Apodemus sylvaticus*, in the cereal ecosystem. *Behavioral Ecology and Sociobiology*, **34,** 337–345.

Thornhill, R. 1976. Sexual selection and nuptial feeding behaviour in *Bittacus apicalis* (Insecta: Mecoptera). *The American Naturalist*, **110,** 529–548.

Todrank, J. & Heth, G. 2001. Rethinking cross-fostering designs for studying kin recognition mechanisms. *Animal Behaviour*, **61,** 503–505.

Todrank, J., Heth, G. & Johnston, R. E. 1999. Social interaction is necessary for discrimination between and memory for odours of close relatives in golden hamsters. *Ethology*, **105,** 771–782.

Tooby, J. 1982. Pathogens, polymorphism, and the evolution of sex. *Journal of Theoretical Biology*, **97,** 557–576.

Trivers, R. L. 1972. Parental investment and sexual selection. In: *Sexual selection and the descent of man 1871–1971* (Ed. by Campbell, B.), pp. 136–179. Chicago: Aldine.

Unger, L. M. & Sargent, R. C. 1988. Allopaternal care in the fathead minnow, *Pimephales promelas*: females prefer males with eggs. *Behavioral Ecology and Sociobiology*, **23,** 27–32.

van Staaden, M. J., Chesser, R. K. & Michener, G. R. 1994. Genetic correlations and matrilineal structure in a population of *Spermophilus richardsonii*. *Journal of Mammalogy*, **75 (3),** 573–582.

vom Saal, F. S. 1984. Proximate and ultimate causes of infanticide and parental behavior in male mice. In: *Infanticide: comparative and evolutionary perspectives.* (Ed. by Hausfater, G. & Hrdy, S. B.), pp. 401–424. New York: Aldine Publishing Company.

vom Saal, F. S. & Howard, L. S. 1982. The regulation of infanticide and parental behavior: implications for reproductive success in male mice. *Science*, **215,** 1270–1272.

von Schantz, T., Göransson, G., Andersson, G., Fröberg, I., Grahn, M., Helgée, A. & Wittzell, H. 1989. Female choice selects for a viability-based mate trait in pheasants. *Nature*, **337,** 166–169.

von Schantz, T., Grahn, M. & Göransson, G. 1994. Intersexual selection and reproductive success in the pheasant *Phasianus colchicus*. *The American Naturalist*, **144,** 510–527.

von Schantz, T., Wittzell, H., Göransson, G., Grahn, M. & Persson, K. 1996. MHC genotype and male ornamentation: genetic evidence for the Hamilton-Zuk model. *Proceedings of the Royal Society of London Series B - Biological Sciences*, **263,** 265–271.

Waldman, B., Frumhoff, P. C. & Sherman, P. W. 1988. Problems of kin recognition. *Trends in Ecology and Evolution*, **3,** 8–13.

Walters, J. 1980. Interventions and the development of dominance relationships in female baboons. *Folia primatologica*, **34,** 61–89.

Warner, C. M., Bronell, M. S. & Rothschild, M. F. 1991. Analysis of litter size and weight in mice differing in *Ped* gene phenotype and the Q region of *H-2* complex. *Journal of Reproduction and Immunology*, **19,** 303–313.

Wedekind, C., Chapuisat, M., Macas, E. & Rülicke, T. 1996. Non-random fertilization in mice correlates with the MHC and something else. *Heredity*, **77,** 400–409.

Wedekind, C. & Füri, S. 1997. Body odour preferences in men and women: do they aim for specific MHC combinations or simply heterozygosity? *Proceedings of the Royal Society of London Series B - Biological Sciences*, **264,** 1471–1479.

Wedekind, C., Seebeck, T., Bettens, F. & Paepke, A. J. 1995. MHC-dependent mate preferences in humans. *Proceedings of the Royal Society of London Series B - Biological Sciences*, **260,** 245–249.

Weir, B. S. 1996. *Genetic Data Analysis II*. Sunderland, Massachusetts: Sinauer Associates, Inc.

Weir, B. S. & Cockerham, C. C. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38,** 1358–1370.

White, P. J. 1986. Female discrimination of male dominance by urine odor cues in hamsters. *Physiology and Behaviour*, **37,** 273–277.

Whitten, W. K. 1956. Modification of the oestrous cycle of the mouse by external stimuli associated with the male. *Journal of Endocrinology*, **13,** 399–404.

Wilkinson, G. S. & Reillo, P. R. 1994. Female choice response to artificial selection on an exaggerated male trait in a stalk-eyed fly. *Proceedings of the Royal Society of London Series B - Biological Sciences*, **255,** 1–6.

Williams, G. C. 1966. *Adaptation and Natural Selection.* Princeton, NJ: Princeton University Press.

Wills, G. D., Wesley, A. L., Sisemore, D. A., Anderson, H. N. & Banks, L. M. 1983. Discrimination by olfactory cues in albino rats reflecting familiarity and relatedness among conspecifics. *Behavioral and Neural Biology*, **38,** 139–143.

Wilmsen Thornhill, N. 1993. *The Natural History of Inbreeding and Outbreeding.* Chicago, London: The University of Chicago Press.

Wilson, E. O. 1987. Kin recognition: an introductory synopsis. In: *Kin recognition in animals.* (Ed. by Fletcher, D. E. & Michener, C. D.), pp. 7–18. New York, Chichester: John Wiley & Sons.

Wilson, W. L., Elwood, R. W. & Montgomery, W. I. 1993a. Infanticide and maternal defence in the wood mouse *Apodemus sylvaticus*. *Ethology Ecology and Evolution*, **5,** 365–370.

Wilson, W. L., Montgomery, W. I. & Elwood, R. W. 1992. Range use in female woodmice (*Apodemus sylvaticus*) in deciduous woodland. In: *Wildlife Telemetry, remote monitoring and tracking of animals* (Ed. by Priede, I. G. & Swift, S.), pp. 549–560. London: Ellis Horwood.

Wilson, W. L., Montgomery, W. I. & Elwood, R. W. 1993b. Population regulation in the Wood Mouse *Apodemus sylvaticus* (L.). *Mammal Review*, **23,** 73–92.

Winn, B. E. & Vestal, B. M. 1986. Kin recognition and choice of males by wild female house mice (*Mus musculus*). *Journal of Comparative Psychology*, **100,** 72–75.

Wolff, J. O. 1985a. Maternal aggression as a deterrent to infanticide in *Peromyscus leucopus* and *Peromyscus maniculatus*. *Animal Behaviour*, **33,** 117–123.

Wolff, R. J. 1985b. Mating behaviour and female choice: their relation to social structure in wild caught House mice (*Mus musculus*) housed in a semi-natural environment. *Journal of Zoology*, **207,** 43–51.

Wolton, R. J. 1984. Individual recognition by olfaction in the wood mouse, *Apodemus sylvaticus*. *Behaviour*, **88,** 191–199.

Wolton, R. J. 1985. The ranging and nesting behaviour of Wood mice, *Apodemus sylvaticus* (Rodentia: Muridae), as revealed by radio-tracking. *Journal of Zoology*, **206,** 203–224.

Wrangham, R. W. 1997. Subtle, secret female chimpanzees. *Science*, **277,** 774–775.

Yamazaki, K., Beauchamp, G. K., Bard, J. & Boyse, E. A. 1996. Odortypes in the governance of social interactions. *Behavior Genetics*, **26,** 602.

Yamazaki, K., Beauchamp, G. K., Kupniewski, D., Bard, J., Thomas, L. & Boyse, E. A. 1988. Familial imprinting determines H-2 selective mating preferences. *Science*, **240,** 1331–1332.

Yamazaki, K., Beauchamp, G. K., Matsuzaki, O., Kupniewski, D., Bard, J., Thomas, L. & Boyse, E. A. 1986. Influence of a genetic difference confined to mutation of H-2^k in the incidence of pregnancy block in mice. *Proceedings of the National Academy of Sciences of the United States of America*, **83,** 740–741.

Yamazaki, K., Boyse, E. A., Miké, V., Thaler, H. T., Mathiesoon, B. J., Abbott, J., Boyse, J., Zayas, Z. A. & Thomas, L. 1976. Control of mating preferences in mice by genes in the major histocompatibility complex. *Journal of Experimental Medicine*, **144,** 1324–1335.

Yamazaki, K., Yamaguchi, M., Andrews, P. W., Peake, B. & Boyse, E. A. 1978. Mating preferences of F_2 segregants of crosses between MHC-congenic mouse strains. *Immunogenetics*, **6,** 253–259.

Zeh, J. A. & Zeh, D. W. 1996. The evolution of polyandry I: Intragenomic conflict and genetic incompatibility. *Proceedings of the Royal Society of London Series B - Biological Sciences*, **263,** 1711–1717.

DANKSAGUNG

Mein herzlichster Dank gebührt natürlich meiner Betreuerin, P.D. Dr. Gabi Gerlach. Liebe Gabi, ich habe es sehr geschätzt, mit Dir zusammen zu arbeiten. Von Anfang an warst Du für mich und die Mäuse da, hast mich immer konstruktiv unterstützt. Ohne Deine hilfreichen Ideen, was man mit Mäusen alles testen kann, wäre ich sicherlich zwischendrin bei den Vaginalabstrichen von unwilligen Mäuseweibchen verzweifelt. Als Statistikfachfrau warst Du nur von W. Nagl zu toppen, dem ich hier gleich auch mit danke. Ich wünsche uns weiterhin gute Zusammenarbeit, weitere gemeinsame Veröffentlichungen stehen ja noch an!!!

Manchmal kommt man recht weit rum im Zuge der Dr.-Arbeit, ich kam immerhin bis nach Hamburg und dort möchte ich Simone (Dr. Simone Sommer) für das Einführen in die komplizierte Welt der SSCP Gele und die nette Aufnahme in ihre Gruppe in Hamburg danken. Für die Einführung in die Mikrosatelliten-Analyse mache ich Tina Harr, unsere Co-Autorin „haftbar“. Damit fing die Waldmaus-Genetik an! Und war sie nicht erfolgreich?

Wenn man Wissenschaft betreibt, muss man irgendwann lernen, dass nicht immer alles klappt wie man es sich denkt. Bei wirklich großem Pech, funktioniert überhaupt nichts. Dies passierte leider bei unserer theoretisch so fantastischen Telemetrie Anlage. Trotz fehlendem End-Erfolg haben Harald Kautz und Harry Richter aus der Abteilung Elektronik über lange Zeit versucht, das Unmögliche möglich zu machen. Herzlichen Dank dafür!

Dank gebührt den Mitarbeiter/innen der Tierforschungsanlage der Universität Konstanz, die unseren wilden Waldmäusen viel Toleranz entgegen brachten und mir vor allem das Waschen der dreckigen Käfige abgenommen haben, eine nicht zu unterschätzende Arbeitserleichterung bei phasenweise 300 Mäusen.

Neben fachlicher und sachdienlicher Unterstützung, schätze ich besonders die persönlichen Kontakte und Unternehmungen, die zum Gelingen des Ganzen beigetragen haben. Unsere gemeinsame Exkursion nach Rovinj mit Kirsten, Andreas (Bally), Gabi und Thomas wird mir speziell wegen der darauf folgenden K&K Verwirrung für immer im Gedächtnis bleiben, die

auch Jahre später noch anhält. Der kleinen, aber feinen AG Gerlach und auch meiner Teilzeit AG Meyer danke ich für das nette Arbeitsklima, ihr Interesse und die vielfältigen Aktivitäten wie Badminton spielen, Wandern, Barbecues, Killer-Caipirinha trinken und dass ich immer mit in die Mensa mitgenommen wurde.

Natürlich haben auch meine Mäuse selbst großen Dank verdient. Sie haben sich willig vermehrt; und so den einen oder anderen Ratschlag bezüglich der Partnerwahl habe ich dann auch beherzigt. Nicht immer der größte ist der beste (Sorry André☺), oder der zu ähnliche Norddeutsche Volleyballer (Sorry Dirk ☺), sondern der etwas fremdere Kanadier (Jippie Tony!). Aber nichtsdestotrotz möchte ich mich bei allen sehr für ihre Unterstützung während unterschiedlicher Phasen der Dr.-Arbeit bedanken. Vor allem Tony hat in der letzten Phase unermüdlich an meinen englischen Formulierungen gefeilt und mich mit Fragen gelöchert und somit viel zum Gelingen der Arbeit beigetragen.

Meinen Eltern danke ich sehr herzlich für ihr immerwährendes Interesse an meiner Arbeit, ihre Hilfe bei der Einrichtung einer varibler Mäusezucht durch selbstgefangene Waldmäuse aus unserem Haus in Jever und vor allem auch durch ihre stete Bereitschaft, mich in „Notlagen“ finanziell zu unterstützen. Und meine Schwester freut sich jetzt sicher, dass ich endlich „Mäusearzt“ geworden bin. Meine Patentante Anne hat finanziell dafür gesorgt, dass ich diese Arbeit jetzt auch Binden lassen kann, vielen Dank!