# MATING BEHAVIOUR AND FEMALE MATE CHOICE IN THE HARVEST MOUSE (*MICROMYS MINUTUS*)

A thesis submitted for the degree of Masters by Research

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## *Abstract*

The harvest mouse (*Micromys minutus*) is Britain's smallest rodent, and one of its least-studied mammals. Mounting concerns over the status of its British populations highlights the paucity of information about this species, not least about different aspects of its behaviour. I study the mating behaviour of the harvest mouse, focusing on female mate choice, and specifically on the effect familiarity with the male has on the female's mating preference.

In order to isolate the role of familiarity on female mate choice, I conducted a controlled choice experiment, in which the female had a choice between two tethered males - one familiar and one unfamiliar to her. The results support the prediction that female harvest mice prefer familiar males over unfamiliar ones when they are in oestrus. Results also reveal that females spend more time in proximity to unfamiliar males when they are not in oestrus, and that the difference in the body weight of the males affects the strength of their response.

A more naturalistic experiment was also set up, in which a female and two males - again one familiar and one unfamiliar – were allowed to interact unhindered. This setup allowed the recording of full mating cycles, including copulations. These experiments provide descriptive information about both inter- and intra-sexual interactions surrounding mating. Dominance relations between the two males were very clear cut and the female mated with the dominant male, and only with him. Familiarity did not seem to affect the female's mating preference.

In summary this study shows that female harvest mice prefer familiar males over unfamiliar ones and that male familiarity and dominance interact to influence female mate choice. The study also provides background information about the harvest mouse's mating behaviour and intra-male interactions with potential significance for the understanding of other aspects of the natural history of this poorly studied species.

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# Chapter 1. General Introduction

## 1.1. Female mate choice

Sexual selection theory predicts that, when it comes choosing mates, females in most mammal species will be the more discriminating sex, as they invest more of their total reproductive effort in each offspring (Trivers 1972).

### *1.1.1. Mate choice - general background*

Being discriminating about potential mates can incur costs - such as loss of time and energy or increased predation risk - but also provides many benefits (Andersson 1994). Some of these benefits include – nutrition provided by the male [mostly in birds (e.g. Green & Krebs 1995; Helfenstein et al. 2003; Wiggins & Morris 1986) and insects (reviewed in Vahed 1998); parental ability [in some fish for example females will spawn more readily in a male's nest if it already contains eggs (see Jamieson & Colgan 1989); in some species of birds male song rates, which might be connected to female mate choice, are correlated with participation in parental care (e.g. Greig-Smith 1982)]; defended resources [in many birds, male mating success increases with territory size or quality (e.g. Buchanan & Catchpole 1997; Dale & Slagsvold 1990)]; and risk reduction [mating with dominant males can reduce the likelihood of interruption by other males (Clutton-Brock & McAuliffe 2009; Trail 1985) and the risk of infanticide (Huck & Banks 1982)].

An important benefit of mate choice is ensuring good genes for the resulting offspring. Several theories have been put forward to explain how females choose mates that have a superior genetic quality. Zahavi (1975) proposed that males advertise their quality by incurring a handicap. By assessing the magnitude of the handicap which the male is able to support, the female can then choose the best quality male. Hamilton and Zuk's hypothesis (1982) more specifically addresses bright plumage in birds, suggesting that these indicate genetic resistance to parasites. By preferring brightly coloured males, females have more successful offspring, as they are more resistant to parasites. This mechanism has since also been suggested for brightly pigmented fish (Houde & Torio 1992) and insects (Zuk (Zuk et al. 1993). Fluctuating asymmetry (FA) – which Soule (Soule 1982; Soule & Cuzinroudy 1982) has proposed is a measure of an individual's ability to develop in the face of environmental and genetic stresses - has been proposed as a characteristic which females may use as an indicator of the overall genetic quality of a male (Møller & Pomiankowski 1993; Watson & Thornhill 1994). Several studies have shown females in some species prefer more symmetrical males (e.g. Møller 1992; Swaddle & Cuthill 1994).

The major histocompatibility complex (MHC) is another mechanism which has been proposed to affect mate choice. Whereas the above mentioned preferences and 'good genes' hypotheses are based on females recognizing an absolutely superior characteristic in male – be it genetic quality or other factors – MHC-led mate choice is based on genetic compatibility. Many studies have shown disassortative mating by MHC haplotype in mice (Potts et al. 1991; Yamazaki et al. 1976), and cues regarding

MHC similarity are known to be mediated by urinary olfactory cues (Brown & Eklund 1994). Studies have also shown an interaction between genetic quality and genetic similarity in the mate choice decisions of female mice (Roberts & Gosling 2003).

### *1.1.2 Mate choice in mammals*

While the study of female mate choice in mammals has been lagging behind studies in the more ornamental classes, mostly birds and fish (Clutton-Brock & McAuliffe 2009), in recent years more and more studies have provided evidence that female mammals choose to mate with males which provide them and their offspring with fitness benefits (Jennions & Petrie 2000; Roberts & Gosling 2003).

Numerous studies highlighted the different factors affecting female mate choice in mammals. Some of these factors are – a preference to mate with dominant males (in rodents - Drickamer 1992; Huck & Banks 1982; Horne & Ylonen 1996; Rolland et al. 2003; Shapiro & Dewsbury 1986; in primates and carnivores female also appear to often favour dominant partners – Jahnson 1984, West & Packer 2002, Sillero-Zubiri et al. 2004); in some rodents, females prefer to mate with unmated males, which will have a higher sperm count (Huck et al. 1986; Pierce & Dewsbury 1991). In many mammals males with higher levels of testosterone are more aggressive and colourful, and females prefer to mate with such males (Setchell & Dixson 2001; West & Packer 2002); whereas in others, vocal displays can affect the female's choice (Charlton et al. 2007; Hiebert et al. 1989; McElligott & Hayden 2001).

Olfactory cues are an important source of information for the female mammal, one which plays an important part in mate choice (Gosling & Roberts 2001). Females use olfactory signals to gather information about different male characteristics such as parasitic load (see Penn & Potts 1998), dominance status (e.g. Gosling & Roberts 2001; Huck & Banks 1982) and the major histocompatibility complex (Brown & Eklund 1994).

Another common form of mate choice in mammals is inbreeding avoidance, with kin recognition playing an important role (Hepper 1986) . In other species – such as the Californian ground squirrel, *Otospermophilus beecheyi*, or primates such as chimpanzees, *Pan troglodytes* (Pusey 1987) - dispersal patterns in which only one of the sexes disperse result in matings between close relatives being unlikely. It is not always clear however, whether inbreeding avoidance was the driving cause for these dispersal patterns to evolve (for a theoretical discussion of the effects of competition and inbreeding avoidance on the evolution of dispersal see for example Motro 1991). In such species it is likely that females will have poor kin-recognition mechanisms (Grahn et al. 1998; Pusey & Wolf 1996).

Familiarity between the female and male is often connected to inbreeding avoidance, as individuals in species in which inbreeding is likely, tend to avoid mating with familiar animals (Clarke & Faulkes 1999; Frynta et al. 2010; Tai et al. 2000). In other species, possibly those in which inbreeding is less likely due to dispersal, females show the opposite preference and tend to mate with familiar males

(Coopersmith & Banks 1983; Daly 1977; Randall et al. 2002; Thompson et al. 1995). But see further discussion in Chapter 2.

The most convincing evidence for the occurrence of female mate choice in mammals come from captive studies, mainly of rodents, where male competition and coercion can be minimised or even excluded (Clutton-Brock & MCAuliffe 2009, and for example – Gubernick & Addington 1994; Ricankova et al. 2007; Roberts & Gosling 2003; Rolland et al. 2003; Thom et al. 2004). And while, in many mammals, the males are larger than females and often use their size to constrain female mate choice (Clutton-Brock & MCAuliffe 2009), in animals in which there is no size dimorphism, females are likely to have more freedom to exercise their choice even in more naturalistic situations. One such animal is the harvest mouse *Micromys minutus* & (Harris & Trout 1991). Female harvest mice are also considered dominant to the males (P. Rudd, Chester Zoo, pers. comm., Trout 1978b), possibly reducing even further the influence of intra-male interactions and male constraints on female choice. This makes the harvest mouse a particularly interesting species in which to study female mate choice.

## 1.2. Mate choice and conservation

While studying mating behaviour and mate choice is interesting in and of itself, this is also relevant in the context of conservation. In the past 15 years there has been a growing recognition of the importance of animal behaviour studies to conservation efforts. Subjects range from understanding the habitat requirements of a species and predicting the consequences of environmental change, to studying mating systems and reproductive behaviour to improve captive breeding and a better understanding of the breeding dynamics of a wild population (Sutherland 1998).

Conservation efforts have much to gain from better knowledge of a species social behaviour, even in a solitary species. In the Stephens' kangaroo rat *Dipodomys stephensi* for example, knowledge of the social interactions within a relocated population is important for the success of the relocation, as animals had a higher survival rate when they were relocated with their neighbours (Shier 2009). And in the Heermann’s kangaroo rat *D. heermanni*, a study of pair formation and oestrous cycle resulted in recommendations for improving captive breeding practices (Yoerg 1999).

One of the most important social interactions in a species, and therefore of potential importance for its conservation, is its mating behaviour. A species’ mating system – which describes the breeding sex ratio – affects the effective population size ($N_e$), which is a sensitive predictor of the ability of a population to maintain genetic variability. This in turn can be translated to long-term viability of the population (Anthony & Blumstein 2000; Bessa-Gomes et al. 2003; Parker & Waite 1997).

In mammals for example, mating systems have an affect on a species' vulnerability to hunting by humans, where monogamous and weakly polygynous species are more vulnerable to hunting of males than species where a dominant male mates with many females (Greene et al. 1998). Promiscuous primates can survive at smaller population densities than do solitary or monogamous ones (Dobson & Lyles 1989), and a similar pattern is found in birds (Legendre et al. 1999). Other aspects of a species reproduction will also affect a population's vulnerability to various forms of exploitation. Reproductive suppression by a dominant female for example will make a small population of such species even more vulnerable (Côté 2003).

Anthony & Blumstein (2000) list nine behavioural traits which influence $N_e$, including the mechanisms of mate choice. As choice implies that some animals are not chosen, and therefore do not mate, the effective population size is reduced and the reproductive skew, and potentially growth rate, of a population are affected. Patterns of mate choice also affect a population's genetics and its risk of extinction (Quader 2005). Better understanding of mate choice decisions in a species can therefore enable conservationists to predict some of the consequences of habitat alterations, population changes and human disturbance.

Mate choice, especially where a 'fixed threshold' mechanism is present (Anthony & Blumstein 2000), or where a female requires a large enough sample of potential mates before making her choice, will increase the vulnerability of an already small population, as females might not breed at all, or mate with lower quality males. The latter could result in the females reducing the number of offspring, or their investment in each one. This process will result in exacerbating the Allee effect (Quader 2005). In elephants for example, removal of the large males from the population (due to hunting) resulted in a very skewed sex ratio which the authors fear will combine with the females' preference for larger males to limit reproductive rates (Dobson & Poole 1998). However mate choice can also negate some of the negative results of a small population. In populations where females choose to mate with non-kin for example, this can balance the increased danger of inbreeding.

Côté (2003) believes that "the study of animal behaviour can make its most significant contribution as a tool for predicting the effectiveness of conservation measures on population sizes." However, captive breeding can also benefit from better understanding of a species' mating system, as applying such knowledge when planning captive breeding programmes can increase their rates of success (Quader 2005; Swaisgood et al. 2000).

Grahn et al. (1998) suggest that it is important to allow mate choice in captive breeding programmes, and that the attempt to increase genetic diversity of the breeding population should not be the only criterion for pairing animals. Studies in domestic pigeons *Columba livia* (Klint & Enquist 1981), and Mauritius kestrels, *Falco punctatus* (Jones et al. 1995), suggest that allowing females to choose between males increases reproductive success. Drickamer et al. (2000) found that female house mice *Mus musculus* had significantly more litters if they were mated with a preferred male than if they were mated with non-preferred ones; sons from those matings were socially dominant to sons from matings with non-preferred males, and both female and male adults built better nests if they were offspring of a mating with a preferred male.

These lessons have been recognised and several studies in recent years have focused on how to optimize captive breeding for conservation. Examples range from the Lompoc kangaroo rats *D. heermanni* (Thompson et al. 1995) to the giant panda *Ailuropoda melanoleuca* (Swaisgood et al. 2000) and also include the harvest mouse (Roberts & Gosling 2004).

As harvest mice are a species of conservation concern (Section 1.3), studying their mating behaviour will contribute background information that could be relevant to future conservation attempts.

## 1.3. The harvest mouse (*Micromys minutus*)

The harvest mouse *Mircomys minutus* is a Palaearctic species, its distribution ranging from England and Wales in the west, across Europe and Asia to Japan in the east (Harris & Trout 1991). It favours tall, dense vegetation and can be found in a variety of habitats – from salt marshes and reed beds to hedgerows and cereal fields (Harris & Trout 1991). In Europe harvest mice are more common in wetlands, and though present in dry habitats as well, from herb bunchgrass steppes to desert steppes, their abundance there is low (Oparin & Oparina 2009). In Britain they are commonly found in dry habitat as well as wetlands (Perrow & Jowitt 1995). Their presence is easy to discern from their characteristic tennis-ball sized aerial breeding nests. The paucity of monitoring – due in part to their small size and mainly utilizing above-ground habitat – leaves some uncertainty as to the status of the harvest mouse in Britain. However, unpublished data (T. Newman et al., cited in Love et al. 2000) provide strong evidence of a reduction in their numbers, as they have been lost from 74% of the monitored sites. And changes in farming practices have greatly reduced the areas available to them, making them a species of conservation concern (Macdonald et al. 1998; Perrow & Jowitt 1995). Though not necessarily reflecting its status in Britain, the harvest mouse is currently - and has been since 2008 - listed by the International Union for Conservation of Nature (IUCN) as a species of least concern, (IUCN 2010).

Harvest mice are omnivorous, eating seeds, fruit, leaves and insects as well as a little fungus, moss, roots and other invertebrates (Dickman 1986) and even eggs of small birds and flesh of other small mammals (Trout 1978b). Adult body weight is on average 6g (range 4-11g) in Britain (Perrow & Jowitt 1995), 9.9g (range 7-14g) in Turkey (Özkan et al. 2003) and ca. 8g in Japan (Ishiwaka & Mori 1999). There is no size dimorphism between the sexes.

Studies indicate that in Britain the home range of the harvest mouse is on average around 350m$^2$ (ranging between 147 – 800 m$^2$), and both Cross (1967) and Trout (1976) found that individual home ranges likely overlap. These figures however are based on limited data, and much remains unknown about the spatial organisation and social structure in natural populations.

Captive observations have shown activity throughout the 24 hours (personal observations; Docherty 2009; Trout 1978b) and field studies suggest that harvest mice tend to be more nocturnal during the summer and autumn, but are more diurnal in winter (Trout 1976, 1978a). Activity rhythms in captivity vary according to the length of daylight under which the mice are kept, and a large variation in activity patterns is found between individuals. Some captive studies show an even level of activity bouts throughout the day and night (Trout 1978b), others show a somewhat heightened level of activity in the dark, though animals were also active for considerable parts of the day (Docherty 2009).

During the breeding season (from late May until October) harvest mice are solitary, and females are possibly even territorial (Trout 1978a). There are, however, no definite reports about where they spend the winter. Ishiwaka et al. (2010) suggested that several individuals may sometimes share an over-wintering ground nest, though this has yet to be confirmed, and Rowe & Taylor (1964) reported many small individual nests found in corn-ricks during the winter and concluded that in corn-ricks harvest mice overwinter singly. At least until mid-20th century thousands of mice were found aggregating in winter refuges such as ricks (Rowe 1958).

Similar to many small mammals in the UK, the harvest mouse breeds during the summer, with populations reaching their highest levels in autumn (Trout 1976). Over-winter mortality is very high which is likely to be due to their small size, making them vulnerable to many predators, but possibly also because of their high metabolic demands – the daily cost of maintenance of a 7-8g harvest mouse is only slightly less than that of a 20g vole or mouse (Harris & Trout 1991; Perrow & Jowitt 1995).

Gestation period is 17-21 days (P. Rudd, Chester Zoo, pers. comm., Trout 1978b). After the young are born the male is not tolerated near the nest and does not participate in taking care of the young (P. Rudd, Chester Zoo, pers. comm., Trout 1978b). Average litter size in the wild in Britain is 5.4 (Harris and Trout 1991) and in captivity 4.7 (Trout 1978b).

The harvest mouse develops quickly, even for its small size, with the young emerging from the nest and starting to eat solid food when 11-12 days old and are weaned by 15-16 days after their birth, at which point – in captivity - they may be chased from the nest by the mother (Ishiwaka & Mori 1999; Trout 1978b). In captivity animals reach sexual maturity when about 40 days old, though the exact age depends on various external factors such as temperature and crowding (Trout 1978b). Commencement of reproduction also seems to depend on the available mates, as anecdotal evidence from captive breeding suggests that females will delay mating if the only males available to them are their brothers (P. Rudd, Chester Zoo, pers. comm.).

An extensive captive breeding programme conducted by Chester Zoo found that harvest mice breed better – the females are more likely to conceive and likely to do so quicker – when two males and two females are initially placed in the same cage. Only one of the two females breed, as one male and one female emerge as dominant to the other individual of the same sex, and they become the breeding pair. The other two animals are then removed from the cage and paired with other mice, as they will not breed with each other (P. Rudd, Chester Zoo, pers. comm.)

In captivity (in cages and in naturalistic enclosures), before mating occurs the male will usually chase the female on and above ground for several minutes. As the female stops, the male attempts to mount and several matings may follow, usually above ground (Trout 1978b). A vaginal plug forms after mating, which the female quickly loses (Trout 1976).

Not much else is known about the mating behaviour – and more specifically mate choice - in *M. minutus*, therefore these questions inherently warrant further studying. As this species is of mounting concern to conservation, it is also important to consolidate background information about different aspects of its behaviour, therefore motivating this study of female mate choice in the harvest mouse.

## 1.4. Objectives and thesis outline

The main objective of this study was to investigate the role of female mate choice in *M. minutus,* and to provide background information about mating behaviour in this species.

Various male traits affect female mate choice (Section 1.1), but I chose to focus on the effect of familiarity for several reasons:

(a) Familiarity is known to have an effect on many species' mate choice (e.g. Clarke & Faulkes 1999; Daly 1977; Fisher et al. 2003; Randall 1991).

(b) Previous studies suggest familiarity affects female mate choice in the harvest mouse (Roberts & Gosling 2004), therefore it was considered a good trait with which to start investigating mate choice in this species.

(c) Familiarity was the most feasible trait to study with the limited stock of animals available, in which testing the effect of genetic relatedness for example, would have been difficult and would have allowed for only a small sample size.

(d) Cross fostering techniques, which are useful in studies of kin recognition and choice, would have been a problem to implement as females are considered to be very sensitive to nest disturbance (P. Rudd, Chester Zoo, pers. comms.)

(e) The paucity of information on other aspects of the species' social interactions, such as the determinants and expression of dominance between males, meant that studying the effect of such interactions required too many preliminary studies that were beyond the scope of an MSc study.

(f) As preference for familiarity is also connected to other aspects of a species' natural history - such as inbreeding avoidance, dispersal, and spatial organisation - it was considered that investigating the role of familiarity in female mate choice might also shed some light on other aspects of this poorly studied species.

In order to investigate the role familiarity had on female mate choice, I conducted a controlled choice experiment, detailed in Chapter 2, in which the female had a choice between two tethered males. This allowed me to isolate familiarity from other factors affecting mate choice. However it did not provide much information about inter- and intra-sexual interactions surrounding mating, therefore a more natural setup was also devised, detailed in Chapter 3. A further motivation for the second set of experiments was to investigate whether the use of proximity as a proxy for mating preference, as was done in the choice experiment, is valid in *M. minutus* as it is considered to be in other species (e.g. in house mice (Roberts & Gosling 2003) and American mink *Mustela vison* (Thom et al. 2004)).

## 1.5. Ethical note

All work was reviewed and approved by the Zoology Ethical Review Panel and was conducted under Home Office licences PPL 30/2318 (2006) and PIL 30/7297 (2006) (though a Home Office licence was not strictly needed for this work). Interactions between animals were monitored and great care was taken to prevent animals from being physically harmed by other individuals during the experiments described in chapters 2 and 3. No animal was harmed during these experiments.

# Chapter 2. Familiarity and Female Mate Choice in the Harvest Mouse

## 2.1. Introduction

### *2.1.1. Familiarity and female mate choice*

Familiarity affects mate choice in many species, but in different ways. In several species of rodents females prefer familiar males to unfamiliar ones (for example - Mongolian gerbils *Meriones unguiculatus* (Daly 1977); brown lemmings *Lemmus trimucronatus* (Coopersmith & Banks 1983); Merriam's kangaroo rats *Dipodomys merriami* (Randall 1991); Heermann's kangaroo rats *D. heermanni* (Thompson et al. 1995); giant kangaroo rats *D. ingens* (Randall et al. 2002); the mound-builder mouse *Mus spicilegus* (Patris & Baudoin 1998); meadow voles *Microtus pennsylvanicus* (Salo & Dewsbury 1995); prairie voles *M. ochrogaster* (Shapiro et al. 1986); golden hamsters *Mesocricetus auratus* (Tang-Martinez et al. 1993)). The same is true in animals other than rodents such as the pygmy loris *Nycticebus pygmaeus* (Fisher et al. 2003) and in the European rabbit *Oryctolagus cuniculus* (Engel 1990). The opposite preference, for an unfamiliar partner, also occurs; notably amongst highly social animals (for example - the mandarin voles *M. mandarinus* (Tai et al. 2000); the naked mole-rat *Heterocephalus glaber* (Clarke & Faulkes 1999); the gray-tailed vole *M. canicaudus* (Boyd & Blaustein 1985) and possibly also in the house mouse, *Mus musculus* (Frynta et al. 2010; Patris & Baudoin 1998)).

Preference for more familiar males is illustrated, amongst solitary species, by female giant kangaroo rats preferring to interact with nearest neighbours and displaying lower aggression levels toward neighbouring males than towards non-neighbours (Randall et al. 2002). In captivity, female Heermann's kangaroo rats are similarly less aggressive to familiar males (Thompson et al. 1995) as are female golden hamsters (Tang-Martinez et al. 1993) and Mongolian gerbils (Daly 1977).

The prevalent interpretation of the adaptive significance of female preference for mating with familiar males is that familiarity is a proxy for quality. In territorial species, familiarity could arise because the male inhabits an adjacent territory, demonstrating his resource holding potential (Rich & Hurst 1998). In non-territorial species, higher rates of scent marking can be associated with male dominance (Brown & Macdonald 1985; Cheetham et al. 2008; Ralls 1971), which is often preferred by females (in rodents - Horne & Ylonen 1996; Huck & Banks 1982; Rolland et al. 2003; and more generally in animals other than rodents - Wong & Candolin 2005). A high rate of encounter of an individual male's scent marks may thus be a proxy for his dominance and therefore quality (Fisher et al. 2003; Gosling & Roberts 2001; Tang-Martinez et al. 1993). Females of wild-caught house mice, for example use presence of scent marking as a reliable signal of male quality (Rich & Hurst 1998). And Daly (1977) suggests that the main purpose of male scent marking in the Mongolian gerbil is to familiarize neighbouring females, rather than to mark territorial boundaries against intruders.

Additional hypotheses to explain female mating preference for familiar males include gaining better access to information about quality (Rich & Hurst 1998), and therefore reduced necessity to invest time in familiarisation (Frynta et al. 2010). Related to this is the possibility that mating with familiar males involves less risk of harmful aggressive interactions than might occur with less familiar males (Frynta et al. 2010).

A preference for unfamiliar males is mostly attributed to inbreeding avoidance in social mammals (e.g. Ethiopian wolves *Canis simensis* (Sillero-Zubiri et al. 1996); naked mole rats, (Clarke & Faulkes 1999); Mandarin voles (Tai et al. 2000); gray-tailed vole (Boyd & Blaustein 1985); and reviewed in Cheetham et al. 2008), where familiar males are likely to be related to the female (Pusey & Wolf 1996).

Female preferences for various male traits are known to change during different stages of the oestrous cycle. Oestrous female laboratory mice *M. musculus* for example, prefer to mate with MHC-dissimilar males, a preference that disappears in dioestrous females (Egid & Brown 1989). A variation in female MHC preferences throughout the oestrous cycle seems to exist in humans as well (Jordan & Bruford 1998). Female white-footed mice *Peromyscus leucopus* prefer odours from males with an intermediate degree of relatedness to them only when they are in oestrus, but not when they are not in oestrus (Keane 1990). And female Asian elephants *Elephas maximus* are more responsive to urine from musth males when they are sexually receptive than when they are not (Schulte & Rasmussen 1999).

### *2.1.2. Familiarity and the harvest mouse*

In this context of familiarity and mate choice, the harvest mouse *Micromys minutus* is particularly interesting. It is thought to be solitary (Rowe & Taylor 1964; Trout 1978a), with individuals occupying neighbouring or partly overlapping home ranges estimated at 300-400 $m^2$ (Trout 1978a). Relative to its 6-8 g body mass, large dispersal movements of up to 100m over a few days have been recorded (P. Rudd, Chester Zoo, pers. comm.; Trout 1976), with individuals settling c. 90m from their natal nests (Trout 1976, 1978a). The risk of inbreeding therefore has not been considered as a particularly relevant factor in the mating system of this species. Insofar as the predominant mating system amongst solitary rodent species appears to be female preference for a familiar male, and considering that preference for unfamiliar males is generally interpreted as a mechanism for inbreeding avoidance, my expectation was that female harvest mice would prefer a familiar male over an unfamiliar one.

The results of a previous study about the effect of familiarity on female mate choice in the harvest mouse (Roberts & Gosling 2004) were somewhat ambiguous and suggested further investigation was required. The authors presented females with a two-way choice between the scent of a familiar and an unfamiliar male, initially preventing the female from accessing the males themselves, thus exposing them only to olfactory cues for the first 10 minutes. The females were then allowed access to the males' chambers, where the males were constrained behind a perforated, transparent divider, and so the females could both see and smell the males, but not directly interact with them, for a further 30 minutes. The results were somewhat paradoxical insofar as females preferred the familiar male when they had access only to olfactory cues, but not when the females could both smell and see him. In a separate experiment the females were paired with a single male, either familiar or unfamiliar, for 40 minutes. In this sequential setup, the female's interactions with the familiar male were less aggressive than with the unfamiliar male. These results suggest that female harvest mice prefer familiar males, but are based on a short interaction and on less aggression rather than on active preference, and are inconclusive for the two-way choice experiment.

*M. minutus* is also an interesting subject for studying female mate-choice, as there is no size dimorphism between the sexes (Harris & Trout 1991), and therefore males are less likely to constrain female mate choice (Clutton-Brock & McAuliffe 2009). Furthermore, the females are considered dominant to the males (P. Rudd, Chester Zoo, pers. comm., Trout 1978b, but see discussion in Chapter 3), perhaps diminishing the influence of male-male interactions on natural female choice even further. In addition, female mate choice in the harvest mouse - and its mating behaviour in general - is poorly known, therefore these questions also inherently warrant further work.

### *2.1.3. Objectives*

The aim of this study was to examine how familiarity with potential mates affects female mate choice in the harvest mouse.

I predicted that–

1) Sexually motivated females will show a preference for familiar males over unfamiliar males.
2) Females which are not sexually motivated – either because they are not in oestrus or because they are encountering other females – will not show any preference, either for a familiar or for an unfamiliar individual.

To reduce inter-male competition and dominance as confounding factors in female choice (Clutton-Brock & McAuliffe 2009), I developed an experimental design which restricted the males' movements in order to prevent them either interacting with each other or harassing the female. I tested oestrous females, expecting them to be motivated to search for a mate, as well as dioestrous females expecting they were not sexually motivated. To further examine female responses to familiarity in a social rather than sexual context - in order to provide a comparison to the role of familiarity in mate choice - I tested females' preference for familiar and unfamiliar females.

## 2.2. Methods

### 2.2.1. Husbandry

The harvest mice used in this study were bred in Oxford University, using stock from Chester Zoo (United Kingdom) and Wildwood Discovery Park (Kent, United Kingdom). Animals were bred with genetically distant individuals as far as possible, in order to keep a healthy, outbred, population. Animals were separated from opposite sex siblings before sexual maturity and from same sex siblings shortly thereafter. Then animals were housed in individual tanks (Ferplast Geo Extra Large 11L 35x23x22 cm) with wire mesh lids until required.

The animal house was kept at a daily photoperiod of 16h light and 8h darkness and a constant temperature of 21 degrees Celsius. Cages containing either females or males were kept on different racks and a partition between them prevented visual contact between males and females. Female mice were always handled before males to avoid females being accidentally exposed to male odour. Mice were fed *ad libitum* with seed mix (consisting mainly of millet and some sunflower seeds) enriched with insect additive (Orlux Insect Patee, Versele-Laga, Deinze, Belgium). Water was provided *ad libitum* from a dropper bottle. Each cage contained an artificial shelter, wood shavings for bedding, straw for nest building, and at least one dried millet spray, suspended from the lid, as a source of food and behavioural enrichment, in terms of food handling and climbing.

In total, 20 females and 31 males were used for this experiment. Each female was tested at most once during oestrus and once in dioestrus. For each individual female the order between the two tests was random. Males were not used with the same female nor paired with the same male more than once, to avoid pseudoreplication. Males' use as the familiar and unfamiliar male was balanced, so that a male did not participate in several trials as only a familiar male nor as only an unfamiliar one.

### *2.2.2. Experimental setup*

The experimental arena was a clear glass terrarium (28x35x58.5 cm) separated into three chambers using opaque plastic dividers. Each divider had a 2x3cm opening which allowed the female comfortable passage (Figure 2.1). The side chambers were randomly assigned in the first trial to the familiar and the unfamiliar male (see Section 2.2.3. *experimental design*). In each trial thereafter the side chambers were switched between the familiar and the unfamiliar males, the whole arena having been cleaned and disinfected using Trigene (Medichem Int., Sevenoaks) following each trial. Other than their inhabitant, the two side chambers were identical, containing a food bowl, a water bottle and a 2:1 mix of clean wood shavings and used shaving from the home cage of the appropriate male. The central chamber contained a water bottle, clean wood shavings, a shelter and straw from the cage of the test female.

The experiment was filmed remotely using three 85mm (1/3 inch) CCD monochrome security cameras, sensitive to infrared with inbuilt LED illuminators, and recorded on VHS tapes at normal speed.

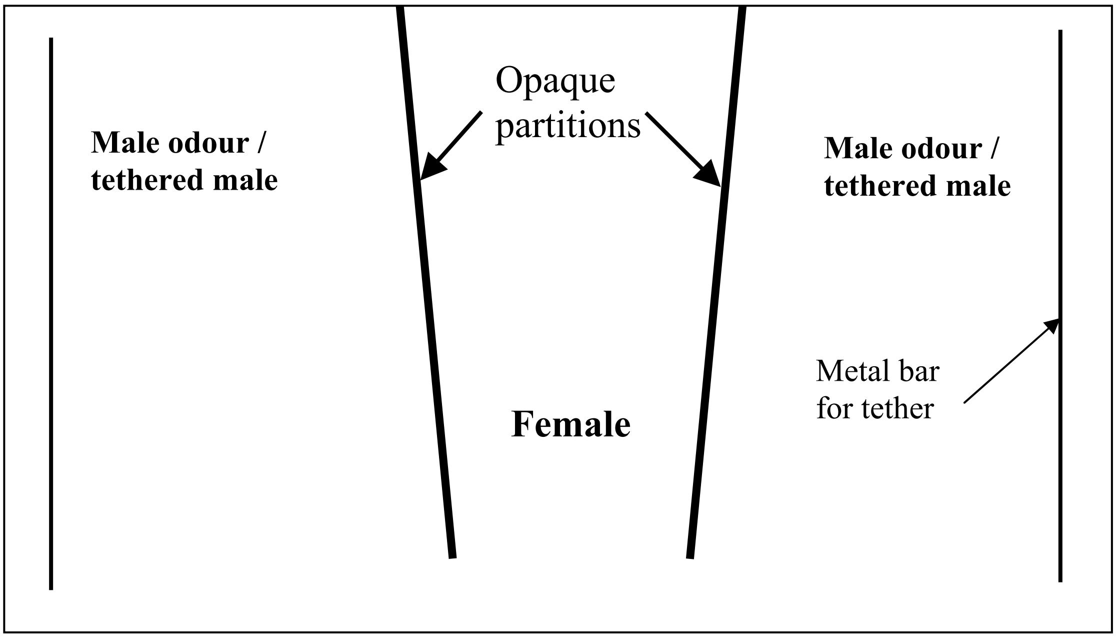

Figure 2.1. The experimental arena (aerial view).

Soiled bedding from the males' home cages were mixed with fresh bedding in the side chambers. For the live preference test, each male was tethered in his respective chamber. Females were placed in the central chamber and allowed to move freely.

### *2.2.3. Experimental design*

Females were checked for oestrous condition by means of a vaginal smear (Freeman 2006; Hubscher et al. 2005; Lefevre & McClintock 1988; Schneider & Popik 2007; Shapiro & Dewsbury 1990) for at least three consecutive days – on the day of the trial, and the two days before (see Appendix). Vaginal secretions were collected using a sterile plastic pipette filled with saline and stained with 0.05% methylene blue. Females were used in an experiment when more than 80% of the cells present in a smear were nucleated or cornified epithelial cells, which characterizes oestrus, or if more than 20% of the cells present were leukocytes, which indicates dioestrus.

For each trial, the two males were paired to be as genetically distant from the female as possible (co-ancestry coefficients between the female and two males ranged between 0 and 0.16, with an average of nearly 0.1; when the coefficient is 0.125 for half siblings and 0.0625 for cousins. Co-ancestry was calculated using a known pedigree) and, secondarily, to minimise body weight differences between them. Test pairs of males also had the same prior experience in terms of number of previous encounters with females and number of times being collared and tethered.

One of the two participating males in each trial was randomly assigned to be the familiar male. 100 ml of loosely packed, soiled bedding (used for 1-2 weeks) was transferred from the familiar male's home cage to that of the test female, beginning at least 6 days before the trial and transferred daily until the day before the trial.

I measured the body weight of all experimental males (in g) to allow me to pair males with minimal weight differences, and once again on the day of their trial to establish their weight during the trial.

The transfer of soiled bedding from the male's cage was intended to mimic the naturalistic reality under which a female would encounter the scent of nearby males, rather than encountering the individual. This is referred to by Cheetham et al. (2008)

as short term familiarity, as opposed to long term familiarity based on social experience. A similar technique of familiarisation was used for example by Zenuto et al. (2007) with tuco-tuco *Ctenomys talarum*.

On the morning of the experiments the males were briefly anaesthetized using an inhalation mixture of oxygen and isoflourane (1.5%) delivered at a rate of 2 $l/min^{-1}$ (Isocare, Animalcare Ltd, York, UK; Mathews et al. 2002) and fitted with collars made from a padded cable tie weighing 0.2g with a diameter of about 35mm. There were then two stages:

Stage 1: odour preference test - the female was placed in the central chamber of the experimental arena, free to move around the entire arena, and filmed for one hour. I completed 15 replicates of this stage with oestrous females, and 15 with dioestrous ones.

At the end of the hour the female was returned to the central chamber, the openings to the side chambers were blocked, and the males were tethered – using a flexible wire with a swivel clip attached to their collars – in their respective chambers. The wire was tethered to a steel rod hanging from the lid of the cage which allowed the males movement throughout their chambers but preventing them from entering the female’s central chamber. The males were left to habituate to the tethering for 45min.

Stage 2: live preference test - following the habituation period, the barriers between the chambers were removed allowing the female free movement throughout the arena. The animals' behaviour was filmed for 4 hours. This stage included 15 trials with oestrous females and 14 trials with dioestrous ones (one dioestrous live trial was removed from the analysis as the female did not enter one of the side chambers at all during this trial).

20 minutes after the start of the live preference test the lights were turned off (in synchrony with the daylight hours of the animal house) and dim red lights were kept on for the rest of trial. Pilot studies had indicated that interactions between the females and the males were more probable in the dark.

*Control – female odour test*

The odour preference test was repeated using scent from familiar and unfamiliar females as stimuli. As intra-female interactions are solely social in nature, this design was used as a control to the male preference experiments, during which the focal female's behaviour could have been a combination of sexual and social motivations, thus highlighting the role of mate preference in the focal female's behaviour. Vaginal smears were usually taken only twice - on the day of the experiment and the day before. Based on the natural history of the species, the risk of aggression between the females caused us not to undertake live preference tests. The control experiment was replicated 11 times with 11 different females – 5 in oestrus and 6 in dioestrus.

### *2.2.4. Analysis*

The number and duration (to the nearest second) of the female visits to each side of the arena was recorded from the video footage. Duration in each chamber was summed and the proportion of time the female spent in the familiar individual's chamber, out of her total time in both side chambers, was used as the dependent variable, after being arcsin transformed to meet the assumption of normality. This proportion of time spent in the familiar chamber was calculated for the entire duration of all trials, as well as for each one-hour bins within the live preference test separately.

Statistical analysis was carried out using SPSS (version 17, SPSS Inc., Chicago). The females' preference was tested using the General Linear Model design to allow to take into account the oestrous state, difference in males' body weight and to weight the data points with the total duration of time the female spent in the side chambers in each trial.

Difference in males' body weight was included in the analysis of the live preference test as the limited number of animals available meant that trials varied in the difference in males' size. As size is a factor that can affect female choice (Andersson 1994; Berteaux et al. 1999), I deemed it necessary to add this variable to the model.

Using the total amount of time the female spent in both side chambers to weight the dependant variable was done as it varied greatly between the trials (e.g. in the live preference test - mean=75.5 minutes +/-59.6; minimum – 7.7 min, maximum – 206.7 min).

## 2.3. Results

### *2.3.1. Effect of familiarity on female mate choice*

Females behaved differently when they were in oestrus and when they were not. Females spent proportionally more time in the chamber of the familiar male when they were in oestrus, and in the chamber of the unfamiliar one when they were in dioestrus (weighted means for proportion of time spent in the familiar male's chamber – in oestrus = 0.154, in dioestrus = -0.155; F (1,27) = 7.68, p = 0.01, partial eta squared = 0.221).

### *2.3.2. Effect of weight difference between the males*

Oestrous state also influenced the response of females to difference in body weight between the two males. Oestrus females spent more time with the familiar male the heavier it was than the unfamiliar one. Dioestrous females spent more time with the familiar male the lighter it was than the unfamiliar one (Figure 2.2). This interaction between the reproductive state of the female and the difference in males' body weight was strong, and is apparent whether the data are weighted [F (1,25) = 22.31, $p < 0.0005$, partial eta squared = 0.472] or not [F (1,25) = 8.99, p =0.006, partial eta squared = 0.264]. This is more clearly shown when presenting the analyses separately for trials when the females were in oestrus or dioestrus [oestrus - F (1,13) = 12.56, p =0.004, partial eta squared = 0.491; dioestrus - F (1,12) = 11.36, p =0.006, partial eta squared = 0.486, data is weighed by total time in males' chambers].

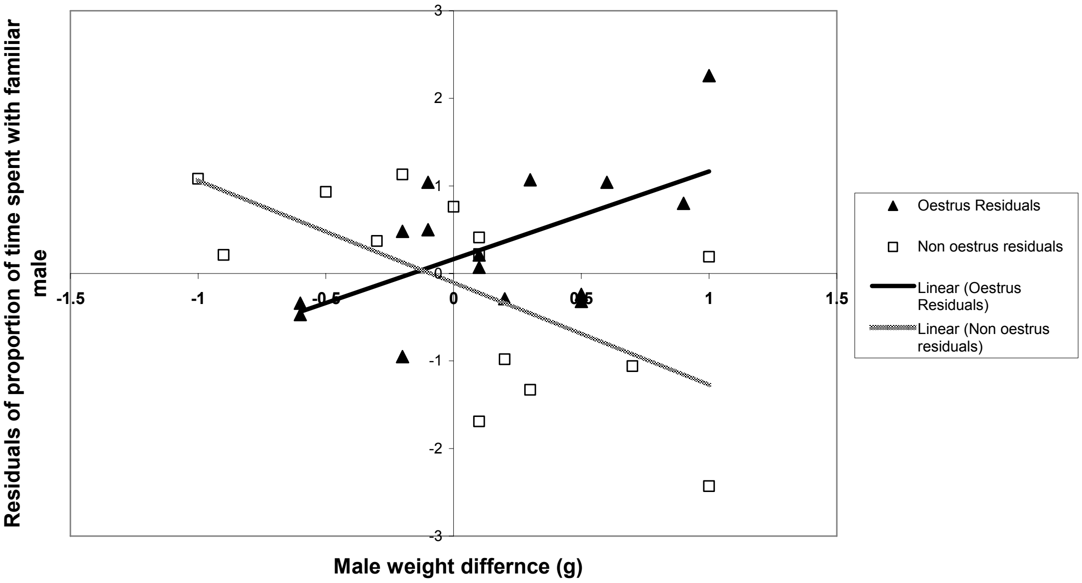

Figure 2.2. Effect of differences in male body weight on proportion of time female spent in familiar male's chamber.

X-axis shows the difference in grams between the weight of the familiar and the unfamiliar male. Y-axis is the residuals of the dependent variable used in the general linear model without female state (oestrus/dioestrus) and difference in male weight.

### *2.3.3. Odour tests*

In both odour tests (stage 1) – either when males' odours were presented to the focal female or in the control, when the odours of other females were used – the focal female did not show a significant preference for either type of scent [male odour test - F (1,28) = 2.44, p =0.129; female odour test - F (1,9) = 0.36, p =0.564]. To investigate whether effects were evident within shorter periods of the four-hour observation, the data from the live test (stage 2) were subdivided into one-hour time bins and analysed in the same way. The third hour alone yielded a significant difference between the behaviour of oestrous and dioestrous females (Table 2.1).

Relatedness (as measured by co-ancestry), age (of the female and the two males), number of days of familiarisation (transfer of bedding) and females' experience (first or second trial participated in), were added to the model but added no statistically significant explanatory power to the females' behaviour.

Table 2.1. Weighted means for proportion of time spent in the familiar male's chamber during one-hour bins of the live preference test compared between oestrous and dioestrous females.

| | **Weighted means – oestrus** | **Weighted means – dioestrus** | **F value** | **P value** |
|---|---|---|---|---|
| **1st hour** | 0.155 | -0.045 | (1,25)=1.932 | 0.177 |
| **2nd hour** | 0.065 | -0.146 | (1,26)=2.638 | 0.116 |
| **3rd hour** | 0.185 | -0.251 | (1,26)=13.92 | 0.001 (partial eta squared = 0.349 ) |
| **4th hour** | -0.065 | -0.254 | (1,22)=1.125 | 0.300 |

## 2.4. Discussion

### *2.4.1. Effect of familiarity on female mate choice*

As hypothesised, my results show that female harvest mice in oestrus preferred to spend more time with a male with whose scent they are familiar, whereas dioestrus females spent more time with an unfamiliar male. Furthermore, this preference also depended on the weight difference between the two males presented to the female. When the female was in oestrus, the larger the weight difference was in favour of the familiar male (i.e. the heavier the familiar male was compared to the unfamiliar male), the more time she spent with him. However, when the female was in dioestrus, the larger the weight difference was in favour of the *unfamiliar* male (the favoured male in this state), the *less* time she spent with him.

The finding that female harvest mice spend more time with the familiar male when in oestrus is consistent with observations of other species of solitary rodents, amongst which females are known to mate with a familiar male, or to be less aggressive towards them (Randall 1991; Randall et al. 2002; Roberts & Gosling 2004; Tang-Martinez et al. 1993; Thompson et al. 1995).

It is common for mammals to devote relatively long times to investigating novel odours (Cheetham et al. 2008). This is in accordance with my finding that dioestrous females spent more time in the chamber of the unfamiliar male. Frynta et al. (2010) pointed out that this tendency for novelty seeking arguably makes preference tests conservative, since for a female to show a preference for a familiar male, she has to overcome her inclination to investigate the unfamiliar one. Therefore, my finding that dioestrous females spent more time with unfamiliar males, if indeed caused by tendencies for novelty seeking, strengthen the significance of the preference they showed for the familiar males during oestrus.

*Using proximity as an indicator for mating preference*

Proximity is often used as measure for mating preference (Egid & Brown 1989; Roberts & Gosling 2003; Thom et al. 2004), and I propose that the time the female spent in the chamber of each male is a good indicator for sexual interest in the harvest mouse as well. In these largely solitary mice, amongst which social interaction appears limited, a non-aggressive association between an oestrous female and a male can plausibly be taken as a proxy for mating preference. Observation of caged animals revealed minimal social interaction – no mutual grooming or other apparent forms of bonding, and adult animals sharing a cage usually keep to themselves.

### *2.4.2. Effect of male body weight*

One hypothesis to explain the effect of male body weight on female choice might be if females can detect, and react to, the size difference. In this case, oestrous females prefer to associate with a heavier male, perhaps as an indicator of quality (see Anderson 1994), whereas dioestrus females, for whom the unfamiliar male is simply an unfamiliar individual rather than a potential mate, may wish to avoid a heavier male, as a potential competitor for resources. To explore this, I tested for any effect of the difference between the female's weight and that of the males, but found none. However, this does not rule out the competitor avoidance hypothesis.

### *2.4.3. Odour vs. live preference test*

In a previous study of mate choice in harvest mice, females showed a significant preference for a familiar male when only odour was available to the female, a preference that disappeared when the female had both olfactory and visual cues (Roberts & Gosling 2004). The authors suggested that their results could be due to visual characteristics having a stronger influence on female choice than familiarity does, though when actual pairs were formed, females showed less aggression towards the familiar male. My results opposed this – no preference was found when only the males' odour was available to the female, but when the males were also present, the preference was significant.

The first stage of the experiment – where only odour was presented – lasted only an hour (to prevent the female getting familiarised with the unfamiliar male's odour before the males were introduced), whereas the second stage, which included the males, lasted for four hours. This could contribute to the difference in the results between the two stages, as Roberts and Gosling's trials lasted either 10 or 40 minutes. Indeed, when the four hours of the second stage were analysed at one-hour intervals, only the third hour showed a significant result. This could indicate that one hour is insufficient to reveal a significant choice in this species. An alternative hypothesis is that the physical presence of the males is the cause of the difference in the results of the two stages of the experiment. These possibilities could be distinguished by a similar experiment, in which the female is given access only to male odour for four hours (rather than to the males themselves).

A possible explanation for the effect of the physical presence of the males on the females' response could be that this is an artefact of the conditions in the animal house. As Salo and Dewsbury (1995) noted, the constraints of laboratory conditions forces some level of exposure to the scent of other individuals in the colony, despite the best efforts to minimize it. Therefore, with some constant level of exposure to male scent, presenting a female with scent alone may not be a sufficient stimulus to effect her behaviour, and the physical presence of the males is needed to influence the female to alter her behaviour in accordance with her preferences.

### *2.4.4. Female odour tests*

The focal females showed no significant preference for odours of a familiar female nor for the odours of an unfamiliar female. However, as the male odour test also did not reveal any preference, whereas the male live preference test did, I cannot rule out that female harvest mice have some preference for spending time either with familiar females or with unfamiliar females. The smaller sample size in female odour test also suggests that further experiments might reveal results that I was unable to obtain here.

### *2.4.5. Effect of oestrous stage on female preference*

The finding that females react differently to familiarity, exhibiting opposite preferences when in oestrous and when not, reflects findings in other species and for other traits (Egid & Brown 1989; Keane 1990; Schulte & Rasmussen 1999); and highlights the importance of conducting mate choice experiments during periods when females are indeed searching for mates. This is likely to be particularly true in species, such as the harvest mouse, in which there is no pair bonding and therefore females do not associate with males during non-receptive stages of the oestrous cycle. Whereas in socially monogamous species, social preferences exhibited by non-oestrous females tend to turn into mating preference when the female becomes receptive (e.g. Gubernick & Addington 1994), there is no reason to expect a similar tendency in animals that are not particularly social.

The results of this study support the hypothesis that female harvest mice prefer mating with a familiar male. Contrary to my original hypothesis, they also show that non-oestrous females spend more time in the unfamiliar males' chambers, possibly due to novelty seeking tendencies. Finally, the results show that females respond to differences in size, both when the male is a potential sexual partner, and when he is a potential competitor.

# Chapter 3. Mating Behaviour in the Harvest Mouse

## 3.1. Introduction

### *3.1.1. Harvest mouse mating behaviour*

The harvest mouse *Micromys minutus* is a small (head and body length - 50-70mm, Harris & Trout 1991), solitary (Rowe & Taylor 1964; Trout 1978a) animal, and studying its behaviour in the wild is therefore extremely difficult. As a result, there are few published studies of general wild harvest mouse behaviour, and fewer still regarding mating.

Female and male harvest mice are not known to associate other than during mating (Trout 1978a), and when kept in the same breeding cage the interaction between them is minimal, mainly involving female aggression towards the male (P. Rudd, Chester Zoo, pers. comm, personal observations). Since multi-male mating (MMM) is known to occur in several other non-monogamous rodents [e.g. house mice *Mus musculus* (Rolland et al. 2003); meadow voles *Microtus pennsylvanicus* (Berteaux et al. 1999); prairie voles *M. ochrogaster*, (Wolff & Dunlap 2002); brown rats *Rattus norvegicus* (Berdoy & Macdonald 2005); red squirrel *Sciurus vulgaris* (Wauters et al. 1990); yellow toothed cavy *Galea flavidens*, (Hohoff et al. 2003)], and benefits to females from multi-male mating have been suggested and documented in rodents as well as other taxa (Clutton-Brock & McAuliffe 2009; Hosken & Stockley 2003; Jennions & Petrie 2000; Stockley 2003; Wolff & Macdonald 2004), it is likely that a female harvest mouse would also mate with more than one male in a particular oestrous period, given the opportunity.

Testes size in the male harvest mouse provides further support to the supposition that multi male mating is likely to occur. Testes are on average 2.28% of body mass of an adult male harvest mouse (Kenagy & Trombulak 1986). This is considered by the authors as relatively large, which suggests male competition, and corresponds to a ‘multi male’ mating system in rodents according to Kenagy & Trombulak’s analysis.

Trout (1978b), in his review of the literature on captive harvest mice, mentions that the male will usually chase the female for several minutes before she stops and mounting attempts take place, followed by several mating events. He suggests that chasing is a regular part of pre-copulatory behaviour and may be an important part of the courtship. In captivity, mating has been recorded to occur on the ground as well as 35mm above ground, on a horizontal platform of stems. While such anecdotal descriptions of captive mating behaviour are sparsely available (Trout 1978b and reviewed literature therein), there is no detailed and quantified description of behavioural interactions associated with mating. There is no information available on the duration of mating interactions, how many mounts and intromissions occur before ejaculation, or how many ejaculates a female receives before being fertilized. Neither is it known how much time is typically required for a female to mate with a cohabiting male, a factor that is potentially important in captive breeding.

Much of the information that is available on mating in harvest mice has come from captive breeding programmes, particularly that led by Chester Zoo. The commonly used protocol, developed for the Chester Zoo programme by Penny Rudd, involves housing two males and two females together. One male and one female emerge as dominant to the other individual of the same sex and they become the breeding pair. The other two animals are then removed from the cage and paired with other mice. This process has been found to promote breeding in the most efficient way, as females are more likely to conceive, and to do so more quickly, in such a setup than when a single female is housed with a single male from the outset (P. Rudd, Chester Zoo, pers. comm.). It is not known whether the female mates with both males, before one of them is removed, or whether she mates only with the dominant male.

Although Rudd's procedure may be effective for captive breeding purposes, and is widely adopted, it is clearly artificial and provides little baseline information about natural mating behaviour in the harvest mouse. This lack of basic information hampers the study of mate choice in the harvest mouse, and in turn restricts attempts to improve their captive breeding (Roberts & Gosling 2004).

### *3.1.2. Role of familiarity with males*

In Chapter 2, I demonstrated a female preference for associating with a familiar rather than an unfamiliar male, when male-male interaction was prevented. However, it remains unclear at what point before mating occurs a female exhibits her preference, whether any such preference is mirrored in social interactions, and how she might interact with a non-preferred male. A further unanswered question is what effect intra-male competition might have on female mate choice.

### *3.1.3. Role of male-male interactions and dominance*

Wilson (1975) defined dominance as “the assertion of one member of a group over another in acquiring access to a piece of food, a mate…or any other requisite that adds to the genetic fitness of the dominant individual”

Many aspects of dominance relationships have been studied, including the effect of age on dominance hierarchies (Berdoy et al. 1995; Borries et al. 1991; Hodgdon & Lancia 1983), context (Hewitt et al. 2009; Macdonald et al. 2002), and the effect of dominance on growth (Schüler & Renne 1988) and access to resources (reviewed in Huntingford & Turner 1987). Another extensively researched area is the role of male dominance in mating behaviour and mate choice (Dewsbury 1982; Qvarnström & Forsgren 1998; Wong & Candolin 2005). Numerous studies have revealed an association between dominance and greater mating success or female mating preference. For example in the brown capuchin monkey *Cebus paella* (Janson 1984), lions *Panthera leo* (West & Packer 2002), the Ethiopian wolf *Canis simensis* (Sillero-Zubiri et al. 2004), fallow deer *Dama dama* (McElligott et al. 2001), guppies *Poecilia reticulate* (Kodric-Brown 1992), and various species of rodents (Carr et al. 1982; Drickamer 1992; Hoffmeyer 1982; Horne & Ylonen 1996; Huck & Banks 1982; Rolland et al. 2003; Shapiro & Dewsbury 1986; White et al. 1984; Wolff 1985).

There are several possible benefits for the female for mating with a dominant male. These include uninterrupted matings, for example in lekking species (ungulates – Clutton-Brock & McAuliffe 2009; birds - Trail 1985); and protection from male infanticide, which occurs in many rodent species (e.g. white-footed mice *Peromyscus leucopus* (Wolff & Cicirello 1989); bank voles *Clethrionomys glareolus* (Ylonen et al. 1997); collared lemmings *Dicrostonyx groenlandicus* (Mallory & Brooks 1978); house mice (Brooks & Schwarzkopf 1983); and meadow voles (Webster et al. 1981)). As dominant males kill young more often than subordinate ones (Huck et al. 1982), by mating with a dominant male, the female reduces the risk of infanticide.

Mating with dominant males also confers genetic benefits. For example, assuming dominance is heritable to a certain extent, sons of dominant males will also be dominant, and therefore have a higher reproductive success (Andersson 1994; Drickamer 1992).

Hypothetically, there are three ways in which the dominance of one male harvest mouse over another might influence which of them mates with a female:

1) Dominance has no effect on female mate choice.
2) Dominance between males determines their mating success. The dominant male prevents, or at least reduces, access of the subdominant male to the female. This will occur if males have some control over mating.

3) Dominance is a factor in female choice. The female may prefer to mate with the dominant male, though she is not physically prevented from mating with the subdominant male.

However, because female harvest mice are believed to be more aggressive than males (P. Rudd, Chester Zoo, pers. comm., Trout 1978b), I `hypothesized that their choice of mate would be little influenced by male-male interaction (as is the case for other female-dominant species, e.g. golden hamster, *Mesocricetus auratus* (Tang-Martinez et al. 1993), giant kangaroo rat, *Dipodomys ingens* (Randall et al. 2002)), and that, when offered a choice of a familiar and an unfamiliar male, females would simply mate with the familiar animal, even if the males were allowed to interact beforehand.

### *3.1.4. Role of female-male proximity*

However if I found that female mate choice was influenced by male-male dominance, my next question would be whether male dominance confers mating success by the dominant male exerting some level of control, or does dominance confer success through its influence on female choice? In other words what is the mechanism for male dominance conferring mating success? This may be addressed by examining which individual is responsible for maintaining proximity to another.

### *3.1.5. Objectives*

In this chapter I attempt to describe the natural history of mating behaviour in the harvest mouse, as well as examining factors in female mate selection, and identifying fruitful directions for future research. More specifically, the aims of this study are:

A. To describe the harvest mouse mating behaviour based on captive observations
B. To examine the role in female mate selection of:
   - Familiarity with the male.
   - Intra-male interactions and dominance.
   - Proximity to the male and whether early proximity indicates mating preference.

### *3.1.6. Ethical note*

All work was reviewed and approved by the Zoology Ethical Review Panel, was conducted under Home Office licences PPL 30/2318 (2006) and PIL 30/7297 (2006) (though a licence was not strictly needed for this work), and adhered to the ASAB code of practice. Measures were taken to prevent animals from being physically harmed by one another during experiments. In particular, aggressive interactions were not allowed to result in injury, and where continued harassment took place, animals were removed from the experiment before aggression escalated to injury.

## 3.2. Methods

### *3.2.1. Husbandry*

The harvest mice used in this study were bred in Oxford University, using stock from Chester Zoo (United Kingdom) and Wildwood Discovery Park (Kent, United Kingdom). Animals were bred with genetically distant individuals as far as possible, in order to keep a healthy, outbred, population. Animals were separated from opposite sex siblings before sexual maturity and from same sex siblings shortly thereafter. Then animals were housed in individual tanks (Ferplast Geo Extra Large 11L 35X23X22 cm) until they were needed for an experiment.

The animal house was kept at a daily photoperiod of 16h light and 8h darkness and a constant temperature of 21 degrees Celsius. Cages containing either females or males were kept on different racks and a partition between them prevented visual contact between males and females. Female mice were always handled before males to avoid females being accidentally exposed to male odour. Mice were fed *ad libitum* with seed mix (consisting mainly of millet and some sunflower seeds) enriched with insect additive (Orlux Insect Patee, Versele-Laga, Deinze, Belgium). Water was provided *ad libitum* from a dropper bottle. Each cage contained an artificial shelter, wood shavings for bedding, straw for nest building, and at least one dried millet spray, suspended from the lid, as a source of food and behavioural enrichment.

### 3.2.2. Experimental setup

The experimental arena consisted of a clear glass terrarium (28 x 35 x 58.5 cm) containing clean wood shavings, one suspended water bottle, two food bowls and several millet sprays for enrichment and to provide the 3-dimensional environment harvest mice are used to. Photoperiod and temperature were as described above for the animal house.

Two variants (A and B) of the experiment were conducted; differences between the two are described where appropriate.

- Type A: trials OX4, OX13, OX14, OX15 and OX20
- Type B: trials OX88 and OX90

Each trial involved one female and two males. The two males for each trial were chosen to be as genetically distant from the female as our stock would allow and, secondarily, to minimise body weight and age differences between the males to reduce the likelihood that females would base their mate selection on differences in these factors. One of the two males in each trial was assigned at random as the familiar male. 100 ml of loosely packed, soiled bedding (used for 1-2 weeks) was transferred daily from the familiar male's home cage to that of the test female, beginning at least 6 days before the trial. To facilitate individual identification, both males in each trial were fur-clipped to reveal their darker under-fur. One male was clipped near the nape, and the other on the rump, the allocation of clip location being assigned at random.

I measured the body weight of all experimental males (in g) to allow me to pair males with minimal weight differences, and once again on the day of their mating trial to establish their weight during the trial. I also recorded their age and the length of time for which they had been separated from their siblings (both in days).

Trials began when the female came into oestrus (this was determined by taking a daily vaginal smear – see Appendix 1 for details on how smears were taken and analysed). On day 1 of the trial the female and two males (one 'familiar' and the other 'unfamiliar' to the female) were placed in the experimental arena simultaneously.

In type A trials, I attempted to monitor the female's oestrus cycle daily throughout the trial. However, taking vaginal smears was disruptive to the interactions under observation (the female became agitated when she was removed from the cage), and I therefore decided not to take vaginal smears in the remaining trials (type B).

The experiment was filmed using three 85mm (1/3 inch) CCD monochrome security cameras, sensitive to infra-red light with inbuilt LED illuminators, and recorded on 4-hour VHS tapes. Filming was continuous, mainly at normal speed, but occasionally at ¼ x normal speed to cover periods when no-one was available to change tapes. Type A trials were filmed using one camera to cover the entire experimental arena. In type B trials, additional cameras were used to film from both sides of the terrarium as well, thereby making sure that subjects were visible at all times (except when they were beneath the bedding), and to allow closer analysis of interactions.

Trials lasted for several days, until the point at which 24 hours had passed since the last mating event (mounting or intromission, following the protocol in Rolland et al. 2003)), or until it was concluded that the animals were not going to mate (e.g. after five days have elapsed).

### *3.2.3. Video analysis*

Video footage of the seven trials was scanned for mating behaviour. In three trials (OX4, OX14 and OX20), no mating behaviour - mounting, lordosis or intromission - was observed, and so these trials were excluded from analysis. The remaining four trials, comprising 234 hours of footage, were analysed as described below.

Video footage from trials OX13, OX15, OX88 and OX90 was observed and interactions (see ethogram in 3.2.4 below) occurring between two or more animals were noted. Interactions from the entire duration of the trial footage were recorded for male-female interactions. Pilot observations on male-male interactions indicated that they were reasonably consistent over time, so male-male interactions were sampled for the first 2 hours of each trial (0-2h) and for the first 30 minutes every 4-hour tape recording thereafter (ideally 4-4.5h, 8-8.5h, etc, but in practice this varied in some cases, e.g. when trials ended or when the length of a recording on a tape varied). This assumption was supported by subsequent analysis of male-male interactions (see Section 3.3.2). For event behaviours (mainly *approach*, *depart* and *lunge*) the time at which they occurred was noted to the nearest second. For state behaviours (see Martin & Bateson 1993, for the distinction between the two types of behaviour) the onset and termination times were noted together with the location (if not on the ground).

All the behavioural data collected were compiled into one-hour bins for the analysis. A one-hour bin was chosen on the grounds that longer periods would mask temporal variation, whereas shorter bins would have resulted in too few events for statistical analysis.

### *3.2.4. Ethogram*

I constructed an ethogram based on my own observations of harvest mouse behaviour. Where appropriate, I referred to Grant & Mackintosh's (1963) and Dewsbury's (1967) definitions for the sake of consistency with the literature. Behaviours are designated either as events (E) or states (S).

**Basic behaviours:**

1. *Approach* (E) - animal comes within one body length of another (this includes accidental approach, when the approaching animal may not be aware of the other individual's presence).
2. *Depart* (E) - animal moves beyond one body length of another, the other animal is stationary.
3. *Sniff* (S) - one animal sniffs another (excluding the ano-genital region).

4. *Nose-to-nose* (S) - noses are in contact or 2-3mm from each other (“nosing” in Grant & Mackintosh, 1963). This behaviour is distinct from mutual sniffing, as both animals tend to be rigid in nose-to-nose.
5. *Genital sniff* (S) - one animal sniffs the ano-genital region of the other.
6. *Hop* (S) - one or more animals hop around the cage, with all four legs repeatedly leaving the ground. Hopping sometimes involves all three individuals and usually ends with the animals in different parts of the cage.
7. *Follow* (S) - one animal follows another in close proximity (less than one body length). Animals are walking, not running.
8. *Paw* (S) - one animal gently touches (or nearly touches, 1-2mm away from contact) another’s face or body with its front paws, usually while sniffing the other. It is most common for a male to paw the female. This is sometimes combined with whole-body sniffing by the male, while moving around the female. In these cases the female tends to stand rigidly. The mouths of both animals are closed.

**Agonistic behaviours:**

9. *Displace* (E) - one animal vacates an area as another approaches, while they remain more than one body length apart. More common, though not unique to, male-male interaction.
10. *Chase* (S) - one animal actively pursues another, and both are running for at least part of the interaction. Chasing usually ends with the animals distant from each other.
11. *Bite* (E) - one animal bites another.

12. *Fight* (S) - any combination of more than one aggressive interaction involving contact between the animals - rolling over each other (always part of a fighting bout), biting, boxing, repeated lunging. Fighting bouts of different intensity and level of aggression were aggregated for simplicity.
13. *Standoff* (S) - animals standing rigid for several seconds at a distance of half to 2 body lengths (not used in analysis).
14. *Box* (S) - Front feet moving quickly, touching or nearly touching the other animal. Mouths are usually open, but no biting. Both animals are usually rigid, and the noses tend to be close to each other. Usually, though not always, boxing behaviour is mutual and more or less symmetric (neither animal is obviously more aggressive than the other). Sometimes both are on their hind legs, using their tails for balance.
15. *Lunge* (E) - One animal lunges once or twice towards the other. Often followed by the other moving away. Sometimes indicates the start of a fight, and therefore the lunging individual is the initiator of the fighting bout.

**Sexual behaviours:**

16. *Attempted mount* (S) – the male places only his front paws on the female's back, or he is trying to mount while not properly 'aligned'. Can include sniffing and/or pawing of the female's back.
17. *Mount* (S) - the male climbs fully on top of the female, his front feet clasping the female's body.
18. *Intromission* (S) – a mount followed by the male self-grooming his genital region; Dewsbury (1967) referred to this as "post-copulatory grooming" ("The

male rests on his haunches and bends his head and body straight down to groom his genitals with his mouth")

19. *Lordosis* (S) – the female takes up a receptive posture. Head and perineum are raised, back is arched, and back feet are planted. Similar to lordosis in other rodents (see for example Dewsbury 1967)

*Related notes*

- When one of the animals was on one of the suspended objects (millet spray or water bottle) and another was beneath, *genital sniff* was difficult to distinguish from regular sniffing. When in doubt, I noted such occasions as regular sniffing.
- I defined a single bout of a specific behaviour as any single activity lasting continuously until a break of more than two seconds.
- At times it was difficult to distinguish between pawing and boxing, especially when filming in ¼ time, and so some behaviours may have been mis-attributed.
- Chase-and-hop and chase-and-fight were sometimes difficult to distinguish, either because they quickly changed from one to the other (chase and fight) or because it was difficult to draw the line between them (chase and hop sometimes seem to be two ends of a continuum). In the analysis chase/hop combinations were treated as chase (as "pure" hopping usually lasts only a short time) and chase/fight combinations were treated as part of a fighting bout when the chasing occurred between two instances of fighting.
- Longer fighting bouts may contain other behaviours, that are not necessarily aggressive (mainly sniffing) if these lasted no more than two seconds in an overall aggressive interaction.

- Nose-to-nose is distinct from mutual sniffing, as both animals tend to be rigid in nose-to-nose. However, in practice, it was difficult to tell nose-to-nose bouts from other variants of sniffing behaviour and therefore, for the analysis, this behaviour was discarded and replaced with either male sniffing (where a male seemed to be actively sniffing the nose of the female, but not the other way round), or mutual sniffing.
- When one of the animals was hidden beneath bedding, an interaction was usually defined as sniffing, unless it was obvious that something else was happening; sniffing was always at least part of the interaction.
- I have assumed intromission when genital grooming followed mounting, as details of harvest mouse copulation are unknown. It is unlikely that every occurrence of post-mounting genital grooming by the male indicates actual intromission, nor that every intromission is followed by such grooming. However, in other rodent species, such as rats, it is considered a reliable indicator (Dewsbury 1967; Hull & Dominguez 2007).
- No behavioural proxy indicating ejaculation is known in the harvest mouse, and I have not seen anything that suggests such a proxy, such as the male freezing for several seconds as happens in the house mouse (Hull & Dominguez 2007).

### *3.2.5. Compiling behavioural data*

#### *Harvest mouse mating behaviour*

Observing interactions in several groups of three individuals over several days produced a large multivariate behavioural data set. A total of 22 different types of dyadic interaction (a certain behaviour between a specific dyad), including amount of time the female spent in proximity to either male, occurred sufficiently frequently for analysis. In order to examine the variety of behavioural responses of the system, I attempted a simple data reduction technique, Principal Components Analysis (PCA), to derive summary axes describing overall patterns among the 22 interaction types. Proximity index was excluded as there were too many missing data. Because two of the non-preferred males were removed from the arena before the end of their trial (see general results) their subsequent interactions with the female were recorded as missing data.

### *Hinde-Atkinson proximity index*

The Hinde (Laudenslager et al. 2010), or Hinde-Atkinson (Roberts & Gosling 2004), proximity index is a measure of which member of a dyad is responsible for maintaining proximity between the two (Hinde & Atkinson 1970). It is the difference between the proportion of approaches and the proportion of departures one individual makes towards the other. Values can range between -1 and +1. If the calculated index is positive, the first individual, individual X, is responsible for maintaining proximity; if the index is negative, it is the other individual, Y, which maintains the proximity between the two. If the index is equal to zero, both animals are equally responsible for maintaining proximity, but if there are no approaches or departures at all, the index is meaningless (not zero).

$$\textbf{Hinde-Atkinson index} = \frac{A_X}{A_X + A_Y} - \frac{D_X}{D_X + D_Y}$$

A – number of approaches
D – number of departures
X, Y – two members of observed dyad

*Role of male-male interactions and dominance*

Dominance was determined by using the 'binomial approach'; for each aggressive interaction (chase, displace, or lunge) and each non-aggressive interaction (approach, sniff, depart) between the two males. One individual was scored as the 'winner' and the other as the 'loser', the winner being the individual doing the chasing, displacing or lunging, or the approaching, sniffing or if the other is departing. For each dyadic pair of males, one was named as 'dominant' and the other as 'subdominant' if one of them won significantly more encounters than it lost, as per Langbein & Puppe (2004).

Responsibility for maintaining proximity between the two males was calculated using the Hinde-Atkinson index for the entire duration of the trial, with the dominant and subordinate males, respectively, substituting X and Y in the above formula.

*Role of female-male proximity*

Time female spent within proximity of another animal (≤ a body length) was calculated either using time of 'depart' minus time of 'approach', or (where 'depart' did not end the interaction) time of the behaviour that did end the interaction (chase, hop, fight or follow) minus time of 'approach'. NB where fight or follow ended the interaction, the end time of the bout was used; where chase or hop ended the interaction the start time was used, because proximity ended when these behaviours began). Similar judgements were made on the few occasions when a bout of proximity began with anything other than 'approach' (usually 'chase').

Responsibility for maintaining proximity was calculated using the Hinde-Atkinson index. To determine whether it was the female or the preferred male that was responsible for maintaining proximity between them, and to test for changes in patterns across the duration of the trials, the Hinde-Atkinson index was calculated for each one-hour time bin, as well as for the total duration of the trial. Averages for the pre-mating, mating, and post-mating periods were calculated using the indices calculated for each one-hour time bin.

The Hinde-Atkinson index between the female and either male was calculated with the female and male, respectively, substituting X and Y in the above formula.

### 3.2.6. Statistical analysis

All statistical analysis was conducted using SPSS (version 17, SPSS Inc., Chicago). As most of the data included in this chapter were not normally distributed, nonparametric tests were mainly used. In most of the statistical tests, the replicates used are units within trials, mainly of one-hour time bins. Inferences from these data are therefore limited as they follow from a small sample size of trials and individuals.

To test whether one male won significantly more encounters than it lost (as per Langbein & Puppe 2004), a chi-square goodness-of-fit test was conducted comparing the observed rates of wins and loses to a hypothetical rate of an even number of interactions won and lost (Section 3.3.4, Table 3.3).

To test which male females spent more time in proximity to, the preferred or the non-preferred, Wilcoxon signed rank test was conducted, comparing average time per hour the female spent ≤ one body length apart from the preferred male and the non-preferred male. Data from the mating period was excluded because by definition the female is likely to have spent more time with the preferred male during mating (Section 3.3.5, Figure 3.2).

To test whether aggression levels between the two males were consistent over time, the rate of the dominant male chasing the subdominant male was calculated for each sampling period. Chasing behaviour was chosen as it was the most pronounced agonistic interaction and was believed to be most representative of aggressive behaviour. Spearman rank correlation coefficient was calculated to test whether any significant correlation existed between the sampling period, as described in Section 3.2.3, and the number of chase events per hour (Section 3.3.4, Figure 3.1).

To explore possible determinants for dominance between each pair of males, a Wilcoxon signed rank test was used to test for a difference in average weight, age and days since separation from siblings between dominant and subdominant males (Section 3.3.4, Table 3.6).

To explore which animal in female-preferred male dyads was most responsible for maintaining proximity throughout the trials, the Hinde-Atkinson index was calculated for one-hour time bins. A one-way between-groups analysis of variance was conducted to explore the differences in the calculated proximity indices for one-hour time bins during pre-mating, mating and post-mating periods for each trial. The effect size for statistically significant results was quantified using eta squared. Post hoc comparisons using the Tukey HSD test were conducted to determine which groups were significantly different. (Section 3.3.5, Table 3.8).

## 3.3. Results

### 3.3.1. General observations

- All four females copulated with only one of the males present in the cage with her. From here on the mated male is referred to as the 'successful' male - both before and after mating has occurred - and the other as the 'non-successful' male.
- No submissive postures were observed. Aggressive behaviour was responded to either with reciprocal aggression (a fighting bout) or by moving away. This seems to indicate that social interaction in the harvest mouse is not very developed, which supports the assumption that harvest mice are solitary species, with only limited interaction between adult individuals.
- Qualitative description of behaviours revealed great variation between trials that is not captured adequately in the summary statistics. For example, all mountings and intromissions in trial OX90 occurred at ground-level, whereas in trial OX88 they occurred almost exclusively above ground-level, on one of the millet sprays (in the other pairs mountings and intromissions occurred mainly, but not exclusively, at ground-level). The female from trial OX15 seemed much less affiliative with the successful male in that trial than other females were with theirs, and she prevented him from mounting for longer than was typical of other females.
- No litters were born as a result of these matings.

- A few anecdotal observations are worth mentioning:
    - On several occasions (and in at least three trial trios), when the successful male was underneath bedding, and the female and non-successful male had been interacting for several seconds, the successful male was observed running towards the interacting pair and chasing away the non-successful male. This may have been a response to vocalization by the non-successful male, the female, or both; I could not perceive any other way that the successful male might have detected these interactions.
    - In some cases animals copulated on the millet sprays in the cage, approximately 25cm above ground.
- The ‘mating period’ was defined as beginning at the one-hour bin when the first lordosis, mounting or intromission occurred, and ending at the time bin in which the last of any of these behaviours occurred. The shortest latency until the beginning of a mating period in any of the four trials was 12 hours.
- The ‘mating periods’ in the four trials were as follows –
    - OX13 – hours 45 to 48 (duration 3h 17min)
    - OX15 – hours 12 to 15 (3h 6min)
    - OX88 – hours 36 to 41 (5h 12min)
    - OX90 – hours 32 to 35 (2h 49min) (one event of lordosis and intromission also occurred during hour 28, but as it was an isolated event, I decided that it did not represent the beginning of the mating period.)

- Two non-successful males were removed from their experimental arena before the end of their trial, to safeguard them from aggression directed towards them mainly by the successful male.
    - OX13 – the non-successful male was removed during hour 23 (before the mating period began)
    - OX88 – the non-successful male was removed during hour 47 (after the mating period ended)

### *3.3.2. Harvest mouse mating behaviour*

Before performing PCA, the suitability of the data for factor analysis was assessed. Inspection of the correlation matrix revealed the presence of many coefficients of ≥0.3. The Kaiser-Meyer-Oklin value was 0.792, exceeding the recommended value of 0.6 (Kaiser 1970, 1974) and Bartlett's Test of Sphericity (Bartlett 1954) reached statistical significance ($p<.0005$), supporting the factorability of the correlation matrix.

PCA revealed the presence of five components with eigenvalues >1, explaining 39.1%, 17.9%, 8.5%, 7.7% and 6.3% of the variance. An inspection of the scree plot revealed a clear break after the second and after the fifth components suggesting that either two or five components should be retained. However Parallel Analysis (Horn 1965), showed that five components had eigenvalues exceeding the corresponding criterion values for a randomly generated data matrix of the same size (22 variables X 234 time bins); this suggested that all five components should be retained. However, because the fifth component explained only 6.3% of the variance and was loaded on only three variables, it was decided to discard it and retain only the first four.

The four-component solution explained a total of 73.2% of the variance (contributions from components 1-4 as given above). To aid in the interpretation of these four components, oblimin rotation was performed (Tabachnick & Fidell 2007). The rotated solution (Table 3.1) revealed the presence of distinct groups, the interpretation of which is detailed in Section 3.4 (Discussion). There were weak positive and negative correlations between the four factors as a result of the rotation (see correlation table, Table 3.2).

NOTE: To confirm that removal of the non-successful male, where necessary, did not affect the overall results, I ran the PCA excluding all variables connected to that male. The results were very similar to those including all variables, with the same three successful-male components extracted, explaining 67.4% of the total variance. The main difference was that the ‘mating’ component explained slightly more variance than the ‘pre-copulation’ component, rather than vice versa as is the case for the full analysis described above (see section 3.4.1 for component names).

Table 3.1. Pattern matrix for PCA with Oblimin rotation of four factor solution of dyadic interactions [a] (S = successful male; NS = non-successful male)

| | PC1 | PC2 | PC3 | PC4 |
|---|---|---|---|---|
| Lordosis | 1.000 | | | |
| Intromission | .862 | | | |
| S Follows Female | .825 | | | |
| Female Sniffs S | .714 | | | |
| S Sniffs Female | .645 | | | |
| Female Approach S | .564 | | | .564 |
| Mount | .439 | | .412 | |
| Female Sniffs NS | | -.985 | | |
| NS Sniffs Female | | -.983 | | |
| Time in proximity to NS | | -.956 | | |
| Female Approach NS | | -.900 | | |
| NS Approach Female | | -.852 | | |
| S Pawing Female | | | .900 | |
| Attempted Mount | | | .784 | |
| S Genital Sniff | | | .637 | |
| Time in proximity to S | .368 | | .616 | |
| S Approach Female | .371 | -.338 | .434 | .307 |
| Female and S Boxing | | | .429 | .426 |
| S and Female Hopping | | | | .735 |
| S and Female Fight | | | | .680 |
| Female lunge at S | | | | .568 |
| S Chases Female | .344 | | | .493 |

Extraction Method: Principal Component Analysis.
Rotation Method: Oblimin with Kaiser Normalization.

a. Rotation converged in 9 iterations.

Table 3.2. Component correlation matrix

| Component | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | 1.000 | -.219 | .324 | .228 |
| 2 | -.219 | 1.000 | -.084 | -.304 |
| 3 | .324 | -.084 | 1.000 | .163 |
| 4 | .228 | -.304 | .163 | 1.000 |

Extraction Method: Principal Component Analysis.
Rotation Method: Oblimin with Kaiser Normalization.

### *3.3.3. Role of female familiarity with the male*

In two trials the female mated with the familiar male (trials OX88 and OX90) and in the other two trials she mated with the unfamiliar male (trials OX13 and OX15).

### *3.3.4. Role of male-male interactions and dominance*

*Interaction outcomes*

Each male was scored as the winner or loser of each aggressive and each non-aggressive interaction using the 'binomial approach' (Langbein & Puppe 2004). In all trials the successful male was the winner of most of the chase and the displace encounters, while lunges were more or less equally conducted by both males in all trials. In all trials the successful male was also the winner in most non-aggressive encounters, although in two trials there may have been insufficient data to detect a significant difference between the successful and non-successful males in sniffing events. The results are presented in Table 3.3.

Table 3.3. Outcome frequencies for dyadic encounters between males over the duration of trials: (a) Aggressive encounters; (b) Non-aggressive encounters.

The first and second columns for each interaction type show interactions in which the Successful (S) and the Non-successful male (NS) were the winner respectively (note that in ‘Depart’, NS departing means P is the winner). A chi-square goodness-of-fit test was conducted in cases where both males acted (i.e. neither cell = 0). Asterisked pairs were significantly different from equality (**p<0.01; *p<0.05).

Table 3.3(a)

| | Chase | | Displace | | Lunge | |
|---|---|---|---|---|---|---|
| Trial ID | S chases NS | NS chases S | S displaces NS | NS displaces S | S lunges | NS lunges |
| OX13 | 24 | 0 | 2 | 0 | 0 | 0 |
| OX15 | 38** | 2** | 12 | 0 | $3^{NS}$ | $4^{NS}$ |
| OX88 | 65 | 0 | 7 | 0 | $3^{NS}$ | $2^{NS}$ |
| OX90 | 91 | 0 | 12 | 0 | 7 | 0 |

Table 3.3(b)

| | Sniff | | Approach | | Depart | |
|---|---|---|---|---|---|---|
| Trial ID | S sniffs NS | NS sniffs S | S approaches | NS approaches | NS departs | S departs |
| OX13 | 12** | 2** | 18** | 6** | 13** | 3** |
| OX15 | 16 | 0 | 51** | 7** | 32** | 3** |
| OX88 | $3^{NS}$ | $1^{NS}$ | 43** | 12** | 26** | 4** |
| OX90 | $4^{NS}$ | $1^{NS}$ | 34** | 2** | 19* | 8* |

*Variation in chase interactions over time:*

In three of four trials the successful male began chasing the non-successful male within the first hour, and in the remaining trial did so within the first two hours (Table 3.4). A successful male was chased by a non-successful male in only one trial (trial OX15), and this occurred just twice in the 12 chase encounters between them.

Table 3.4. Frequency of chase events between successful (S) and non-successful males (NS).

| Trial ID | Hour | S chases NS | NS chases S |
|---|---|---|---|
| OX13 | 1 | 1 | 0 |
| | 2 | 1 | 0 |
| OX15 | 1 | 0 | 0 |
| | 2 | 10 | 2 |
| OX88 | 1 | 2 | 0 |
| | 2 | 1 | 0 |
| OX90 | 1 | 1 | 0 |
| | 2 | 10 | 0 |

To check whether male-male interactions were consistent over time I examined whether chase rate (number of events $hr^{-1}$) by the successful male varied over the course of the trial. Spearman's rank test found no correlation between chase rate and sampling period (OX13, OX15, OX88, OX90; Spearman's rho=-0.2, -0.46, 0.67, 0.15; P=0.8, 0.25, 0.15, 0.73 respectively) indicating that there was no pronounced decrease or increase in the chase behaviour between the start of the trials and their completion.

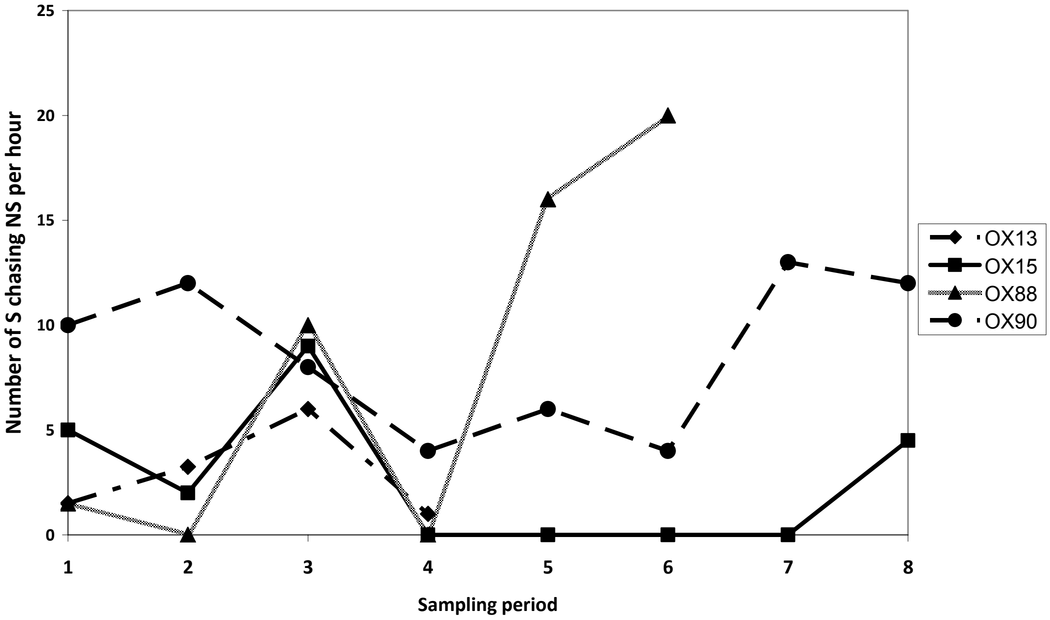

Figure 3.1. Number of intra-male chase events per hour across sampling periods. Successful male chasing non-successful.

*Instigating interaction*

Proximity indices were calculated for each dyad using ‘approach’ and ‘depart’ events (Table 3.5). In each of the four trials the successful male was most responsible for proximity to the other male.

Table 3.5. Proximity indices between the two males in each trial.

A positive value means the successful male is responsible for maintaining the proximity.

| Trial ID | Proximity index |
|---|---|
| OX13 | 0.5625 |
| OX15 | 0.7936 |
| OX88 | 0.6485 |
| OX90 | 0.6481 |

*Role of basic biological factors*

I examined whether body weight, age or time of separation from male siblings had any effect on whether a male was dominant or subdominant during the mating trials. None of these potential predictors had a significant effect on dominance (Table 3.6).

Table 3.6. Basic biological factors of dominant and subdominant males.

Mean (and SE) values for male body weight (g), age (days) and time since separated from male siblings (days). Wilcoxon Signed Rank Test indicated that none of these were related to dominance status (n=4).

| | Dominant | Subdominant | Z | P Value |
|---|---|---|---|---|
| Weight (g) | 5.98 (±0.38) | 5.90 (±0.21) | -0.184 | 0.85 |
| Age (days) | 140.50 (±22.25) | 131.75 (±4.40) | 0 | 1.00 |
| Separation from siblings (days) | 81.25 (±9.1) | 71.5 (±10.96) | -0.37 | 0.72 |

### *3.3.5. Role of female-male proximity*

*Female proximity to successful and non-successful males*

Overall the female spent marginally significantly more time in proximity to the successful, than to the non-successful, male outside of the mating periods (Wilcoxon Signed Rank test, P=0.068, Figure 3.2).

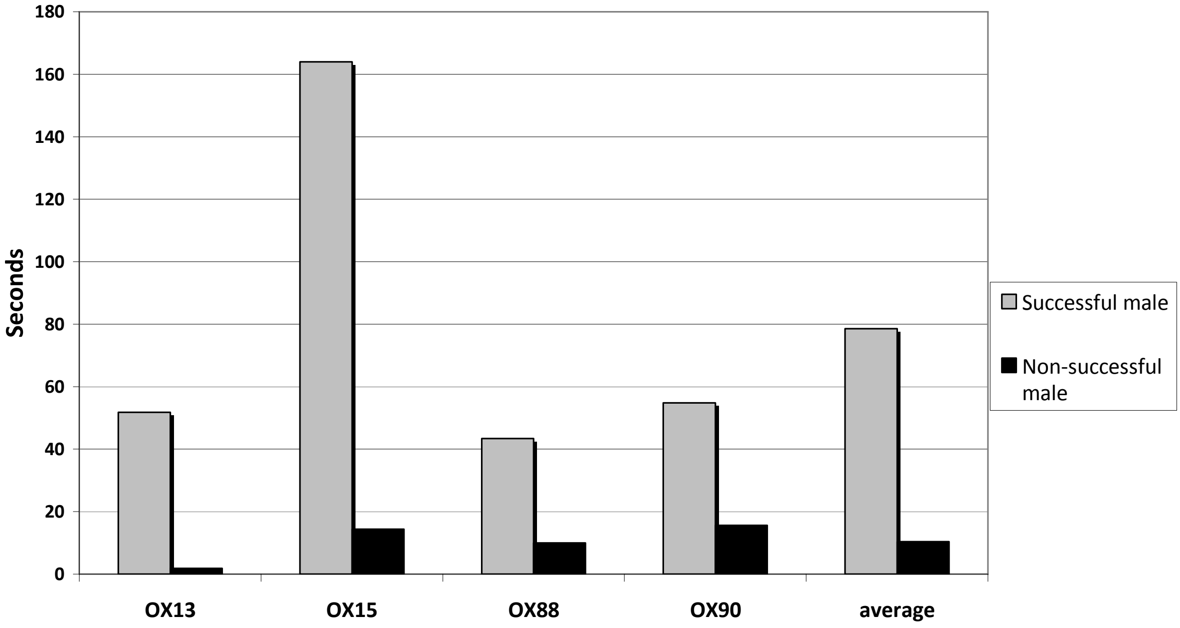

Figure 3.2. Mean time per hour spent by the female ≤ one body length apart from the successful male and the non-successful male.

Data from the mating period was excluded because by definition the female is likely to have spent more time with the successful male during mating.

In three out of four trials the female spent more time during the first hour in proximity to the non-successful rather than the successful male (Table 3.7). In two such cases (OX15, OX88) this changed dramatically during the second hour, such that the female spent more time in proximity to the successful male. In the third case (OX90), the female continued to spend more time in proximity to the non-successful male during the second hour, although the difference between males was smaller, as she spent more time with the successful male than she had in the first hour, and less with the non-successful male. This trend continued in subsequent hours, so that in the third hour the female spent 238 seconds in proximity to the successful male and only 46 seconds in proximity to the non-successful. In the fourth trial (OX13) the female spent more time with the successful male in both the first and second hours.

Table 3.7. Proximity between the female and two males.

Duration of proximity between the female (F) and each of the successful (S) and non-successful (NS) males, and Hinde-Atkinson proximity indices for the female with each of the P and NP males. (XX denotes an incalculable proximity index, as there were no approaches or departures)

| **Trial ID** | **Hour** | **Time F in prox to S** | **Time F in prox to NS** | **Prox index F / S** | **Prox index F / NS** |
|---|---|---|---|---|---|
| OX13 | 1 | 454 | 15 | -0.107 | 0.833 |
| | 2 | 60 | 0 | 0.125 | XX |
| OX15 | 1 | 5 | 36 | 0 | 0 |
| | 2 | 567 | 5 | -0.154 | 0 |
| OX88 | 1 | 14 | 37 | -0.833 | -0.083 |
| | 2 | 50 | 9 | -0.667 | -0.25 |
| OX90 | 1 | 7 | 69 | 0 | 0.5 |
| | 2 | 32 | 52 | -0.5 | 0 |

Proximity indices allowed me to examine whether the female or the male was responsible for maintaining proximity between them. Index values indicated that, during the first two hours, the successful male was more responsible than the female (negative values for five out of eight one-hour bins) for maintaining proximity between them. In three of four trials, either the female was responsible for maintaining her proximity to the non-successful male (positive values), or responsibility was more or less even, during the first two hours.

Looking at the overall proximity indices between the female and the successful male for the entire duration of trials ('total' in Table 3.8), the successful male was responsible for maintaining his proximity to the female (negative index value) in three of four trials. In trial OX90 the male and female were more or less equal in their efforts to maintain proximity with each other. However, in all four trials, the mating period was characterised by a stronger male bias than either pre- or post-mating. Most of these differences are not statistically significant (OX13, OX15, OX88, OX90; *F* (2,48)=4.5, p=0.02; *F* (2,25)=1.2, p=0.33; *F* (2,42)=1.4, p=0.25; *F* (2,41)=1.1, p=0.36. The effect size for OX13 is large8, eta squared = 0.19. Post hoc comparisons for OX13 indicate that mean score for the pre-mating period was significantly different from the post-mating period, p=0.01. The mating period did not differ significantly from either pre or post-mating periods [p=0.99; 0.22 respectively]).

Table 3.8. Proximity index between the female and the successful male.

Negative values indicate that the male is responsible for maintaining proximity.

| **Trial ID** | **Total** | **Average pre-mating period** | **Average mating period** | **Average post-mating period** |
|---|---|---|---|---|
| OX13 | -0.285 | -0.361 | -0.407 | -0.114 |
| OX15 | -0.137 | -0.093 | -0.241 | -0.122 |
| OX88 | -0.089 | -0.148 | -0.152 | 0.183 |
| OX90 | -0.009 | 0.116 | -0.211 | 0.075 |

## 3.4. Discussion

In this chapter I described observations on harvest mouse mating behaviour, about which little has previously been recorded either in captive mice or in natural populations. I then examined the role of female familiarity with the male on mate choice by female harvest mice, as well as male-male interactions, male dominance, and female-male proximity.

Female mate choice could not be explained by female familiarity with the male. In all trials females mated with just one of the two males available. The successful male was the conspicuous winner of most of the chasing and the displacing encounters with the non-successful male, as well as the non-aggressive encounters between them, such as approaching and sniffing. The successful male began chasing the non-successful male within the first 1-2 hours of the beginning of all trials. The successful male chased the non-successful male consistently throughout all trials. Over the whole of all trials only one non-successful male chased the successful male and this occurred only twice out of 40 chases between them. In all trials the successful male was responsible for instigating the majority of periods of proximity with the non-successful male, and for maintaining that proximity. Overall the female spent more time in proximity to the successful, than the non-successful, male. However there was a tendency for the female to spend more time with the non-successful, than the successful, male at the first hour of the trials. This changed to a tendency to spend more time with the successful male by the second hour. During the first two hours of trials, successful males were generally responsible for maintaining their proximity to the female, while the female was responsible in most cases for maintaining her proximity to the non-successful male. Overall the successful male was largely responsible for maintaining his proximity to the female, and during the mating period the intensity of his efforts to be close to female were greater than in either the pre- or post-mating periods. As the data set in this study consisted of only four females and four male-male dyads, the inferences made here are limited and should be verified with further research.

### *3.4.1. Harvest mouse mating behaviour*

Principal Components Analysis of behavioural interactions between the female and each of the males identified four main components which were named according to their biological significance.

Principal Component 1 (PC1) was loaded strongly for the two behaviours specific to copulation, lordosis and intromission. Therefore this component was labelled the 'copulation' component. PC1 also loads strongly for the successful male following the female. This, together with the fact that male following loaded very weakly on the other factors, indicates that this behaviour is closely associated with copulation and that male following may be part of the mating sequence. Male following of the female is common in the mating sequences of other species (e.g - grey wolves *Canis lupus* (Derix et al. 1993); and ungulates such as Kirk's dik-dik *Madoqua kirki* (Kranz 1991)) and not least in rodents (Grant & Mackintosh 1963), but has not been described before in the context of harvest mice.

PC2 was loaded strongly for all five interactions between the female and the non-successful male – female approaching, male approaching, female sniffing, male sniffing and time in proximity - and was labelled the ‘non-successful’ component. The high loading on each of these variables reveals that they are correlated; meaning that during periods when the two animals interacted, both instigated similar levels of these interactions.

PC3 was loaded for precursors to copulation - attempted mounting and genital sniffing by the successful male - and was labelled the ‘pre-copulation’ component. The behaviour of the successful male pawing the female was particularly strongly loaded in this component, suggesting that male pawing of the female is also a part of courtship. However, because this component explains only 8.5% of the variance in the dataset, the possible connection between male pawing and other courtship behaviours, which these results point to, needs to be verified in future studies.

PC4 was loaded largely on behaviours related to aggression between the female and the successful male - hopping, fighting, chasing and female lunging – and was labelled ‘agonistic’. The fact that both hopping and chasing load strongly on the ‘agonistic’ component, but only weakly on others, suggests that these behaviours are connected more closely to aggression rather than being directly related to courtship or copulation. This appears to be contrary to previous suggestions based on anecdotal evidence on harvest mice (Trout 1978b). However, as this component explains only 7.7% of the variance in the data, care must be taken when applying these results to harvest mouse behaviour in general.

Having observed that male-female chasing is more frequent during breeding time, Trout (1978b) and P. Rudd (Chester Zoo, pers. comm.) have suggested that these chases are an element of courtship. My findings seem to contradict this, as chasing was more closely related to other agonistic interactions. However, I propose that the agonistic behaviours observed in these trials are motivationally connected to mating, and distinct from intersexual aggression occurring during non-reproductive periods. Reports from captivity indicate that – unlike the behaviour observed in this study - agonistic behaviour outside the context of mating can be intense, and that it is usually the female which chases the male. In some cases females have been observed to kill males (P. Rudd, Chester Zoo, pers. comm.). It may be that the female's perceived dominance and heightened level of aggression in captivity are artefacts of the fact that most observations previously made were probably made around parturition and during lactation (as they were of breeding pairs), conditions which are known to increase aggression levels in female mammals in general (Maestripieri 1992) and in other rodent species in particular (brown rats *Rattus norvegicus*, (Erskine et al. 1978), golden hamster *Mesocricetus auratus* (Giordano et al. 1984); laboratory and wild house mouse *Mus musculus* (Hurst 1987; Paul 1986)).

The latter proposition is supported by the fact that most documented reports of female aggression towards males occur shortly after mating (Trout 1978b), and by Frank's (1957, reviewed by Trout 1978b) observations of a captive colony in which during breeding time the females were very exclusive of other adults, both males and females, whereas outside reproductive times they showed a high degree of social tolerance. However Docherty (2009), in controlled experiments, found that non-breeding females exhibited more aggression than did males, and that female residents were more hostile towards female intruders than toward male ones, and more so than male residents were to male intruders. Clearly the issue of whether female harvest mice are dominant to males and more aggressive needs further research if the role of female mate choice in harvest mouse mating is to be understood, as intersexual dominance affects the balance between female choice and male coercion. My research has made clear that the next step is to understand how, if at all, dominance amongst the males affects the females' mate choice.

Behaviours appearing in more than one component:

- *Mounting* – was loaded on two components, 'copulation' and 'pre-copulation'. This is to be expected, as mounting without intromission can be considered a stage in an escalating courtship which culminates in copulation.
- *Boxing between the female and the successful male* – boxing behaviour was approximately evenly divided between the 'pre-copulation' and the 'agonistic' components. This is probably more a reflection of the difficulty in differentiating between boxing and pawing than it is of this behaviour being both antagonistic and directly related to courtship.

*Latency to start of sexual interaction*

Many rodent species in which mating behaviour is studied in captive conditions exhibit mating-related behaviour very quickly, sometimes within minutes of being placed in the same arena (in Mongolian gerbils *Meriones unguiculatus* the average latency until first mounting ranged between 58 and 121 seconds (Tang-Martinez et al. 1993); in prairie voles first intromission occurs less than 5 minutes after test started (Shapiro & Dewsbury 1986); in bank voles first ejaculation occurred on average 848 – 1920 seconds after pairs were first allowed to interact (Horne & Ylonen 1996)). This is not the case with the harvest mouse, which does not show any sexual behaviour during short encounters even when the female has been familiarized with the male's scent beforehand (up to 40 minutes in Roberts & Gosling 2004, and four hours in the encounters described in Chapter 2). This study shows that sexual interactions only appeared after a minimum of 12 hours, and in most cases longer than that. Intromissions were also relatively few and far between compared to other rodents which have been more often used for studying mate choice (in Mongolian gerbils 5 intromissions were observed in 15 minutes of interaction (Tang-Martinez et al. 1993); in bank voles up to 115 pre-ejaculatory intromissions were observed in 30 minutes of interaction (Horne & Ylonen 1996)). Even in the trial that had the most intromissions – OX90 –only 14 were observed within a period of 2h 50min. This is despite the fact that trials started when females were in vaginal oestrus, which should have increased their receptivity. Horne & Ylonen (1996) hypothesized that a reason for speedy sexual interactions in rodents is to reduce the risk of infanticide during postpartum matings. As female harvest mice appear to have postpartum oestrus (personal observation of captive breeding), it is possible that the risk of infanticide in this species is low, and therefore the selective pressure to minimise the duration until copulation is weak.

*Lack of successful mating*

There is no conspicuous reason for none of the observed copulations resulting in pregnancy, as females in other breeding pairs in the colony got pregnant 1-3 days after first being housed with a male (although some were pairs previously coupled in one of the trials described in this chapter, so they were already familiar with each other when placed in a breeding cage). It may have been that the whole experimental process (vaginal smears, transfer between cages, perhaps even the red lighting) was too stressful and either prevented successful copulation, meaning there were no ejaculations (which would also explain why I could not see any behaviour that would indicate ejaculation occurring) or caused foetal resorptions, which is known to be – in some rodent species - correlated with social stress (Huck et al. 1988).

### *3.4.2. Role of female familiarity with the male*

Female familiarity with a male did not explain her choice of mate between a familiar and an unfamiliar male. This was unexpected, as in Chapter 2 I found that the females prefer the familiar males, as is the case in some other rodent species (Coopersmith & Banks 1983; Daly 1977; Patris & Baudoin 1998; Randall 1991; Randall et al. 2002; Salo & Dewsbury 1995; Shapiro et al. 1986; Tang-Martinez et al. 1993; Thompson et al. 1995). It is possible that intra-male interactions affected the females' choice and overshadowed their preference for familiar males. This is somewhat supported by the results that in some of the trials the female spent more time in proximity to the subdominant male in the first hour, but later spent much more time in proximity to the dominant male. Her time spent in proximity to the dominant male was probably as a result of a combination of the dominant male actively approaching her and staying in her vicinity, while also chasing the other male away from the female. This suggests that the dominance relations developed between the males prevented the females from mating with their initially successful male.

### *3.4.3. Role of male-male interactions and dominance*

I set out to determine whether interactions between males, and any dominance relationship between them, played a role in female mate choice. Throughout the trials, aggressive and non-aggressive encounters between males were conspicuously one-sided, with one male winning the majority of contests between the two, and the same male had all the sexual interactions with the female. In contrast, lunge behaviours – which were previously assumed to be solely offensive - were evenly balanced between the two males suggesting that they may be partly associated with defensive behaviour.

The high proximity indices between the males suggested that the successful male was largely responsible for maintaining proximity with the other male, and the other male may have been trying to avoid him. This is supported by the fact that overall non-successful males performed 83% of the departures, but only 16% of the approaches (Table 3.3a). This is another indication of asymmetry in relationships between pairs of males in all of the trials, and the dominance of the successful males.

Chasing of the non-winning male by the winner continued throughout the trial, which seems to suggest that although one male was clearly dominating the arena, there was no established hierarchy between the two males, as the dominant male seems to have had to reassert his dominance over and over. Studies in other rodent species show that when hierarchy relations stabilise aggression levels diminish (e.g. brown lemmings *Lemmus trimucronatus* (Huck & Banks 1982); laboratory mice (Fano et al. 1997)). In brown lemmings most attacks and chases occurred within the first three minutes of males meeting, after which subordinate males tended to avoid dominant individuals. In the experiments presented here, aggressive behaviour directed towards the subdominant male persisted throughout the trials (although it did not usually increase in magnitude, when it was suspected that aggression levels increased beyond what is ethically acceptable, subdominant males were removed from the experimental arena).

Failure among males in this study to establish a clear dominance relationship is likely to reflect the natural responses of the species rather than a product of the artificial situation. There were no submissive postures from the subdominant male (and there is no evidence in the literature for such behaviours in the species), which would indicate that in natural conditions they are not required, and no ‘ritualized’ aggressive interactions, in a sense that any obvious injury-limiting strategies were involved (Fano et al. 1997). These types of interaction usually lead to a stable dominance hierarchy which, once formed, reduces aggression and limits the risk of injury to both parties. My findings could indicate that harvest mice have a poorly developed social system, in which a dominant male has no need, or opportunity, to maintain his dominance over other males other than when mating is at stake. This concurs with the fact that wild harvest mice are solitary for most of the year (Rowe & Taylor 1964, Trout 1978a), as some solitary species tend to have a less complex communication repertoire than closely related social species (Schleich & Busch 2002).

While no conspicuous hierarchy was established between the males, one individual consistently ‘won’ interactions with the other throughout the trial. This suggests that the winning male had some inherent attribute which placed him in the winning position. However none of the basic biological factors investigated - male age or body weight - provided an adequate explanation, as no significant difference in these factors was present, though both are known to affect dominance hierarchies (in male laboratory mice heavier individuals won more fights than lighter ones, (Schüler & Renne 1988); larger meadow voles, *M. pennsylvanicus*, are dominant over small ones, (Turner & Iverson 1973) in wild brown rats, *R. norvegicus*, dominance increased with age, independently of weight (Berdoy et al. 1995)).

Contrary to body weight and age, for which the males were selected so that differences between them in these attributes were minimal, asymmetry between the two males in terms of the female’s familiarity with each was designed, and resulting differences in her interaction with each male were possible. However, this asymmetry does not provide a satisfactory explanation either, as in two trials the dominant male was the male familiar to the female, and in two trials it was the subdominant which was the familiar male.

A possible explanation for how dominance was determined in these trials could be whether a male had previously assumed the role of dominant or subordinate (winner-loser effect, Fano et al. 1997; Hock & Huber 2009; Huntingford & Turner 1987). Such a history could only have occurred before they were separated from their male siblings, as this was the only time during which they were housed with other individuals prior to the experiment. However, the males were separated from their siblings a long time prior to participating in an experiment (between 39 and 103 days), whereas in the literature the effect is usually discussed as lasting a few days at most (up to three days in laboratory mice - Fano et al. 1997; the effect of social defeat on behaviour was apparent for up to seven days following that defeat in laboratory rats, which was considered to be a long term effect – Meerlo et al. 1996). Moreover, though a different length of time in isolation can affect the outcome of a confrontation (in pumpkinseed sunfish for example individuals with no recent social experience tend to defeat recent losers, Beacham 1988), there was no significant difference in the length of time since separation from siblings (Table 3.6). This explanation therefore appears improbable.

Another intriguing possibility is that asymmetries in the length of the males' tails – due to nipped ends - can help determine dominance. As the harvest mouse's tail is prehensile (Ishiwaka & Mori 1999) and is important in 3-dimentional mobility, it is possible that a damaged tail affects its owner's performance in agonistic interactions. Docherty's study (2009) showed that females with full tails were more aggressive and less defensive than females with shortened tails. It is unclear whether this is the cause (i.e. because an individual is disadvantaged by the lack of a whole tail) or the effect (the tail is nipped because that individual lost previous encounters).

However, out of the four dyads in this study, only one male had a shortened tail. This probably reflects the fact that the males used here were housed individually since they were separated from their siblings, as usually shortened tails are common in captive bred harvest mice. This single male was in trial OX88 and was the unfamiliar and non-successful (and thus subdominant) male, which supports the hypothesis that the integrity of the tail can affect dominance, but is merely anecdotal.

Of course, the experimental conditions involved may have influenced the importance of social interactions, both in terms of male-male interactions and in how they may have affected female mate choice. Confinement made it relatively easy, for example, for the dominant male to prevent the subdominant from accessing the female, thus creating a situation of 'privileged access' to the females (sensu Dewsbury 1979), as has been recorded for other rodents including deer mouse *Peromyscus maniculatus* (Dewsbury 1979) and brown lemming *L. trimucronatus* (Huck & Banks 1982). Rowell (1974) pointed out that hierarchies witnessed and studied in captivity may, to a greater or a lesser extent, be a result of the limiting conditions and heightened competition (over food, home range and mates) that captive animals are likely to experience compared to their wild counterparts. This, according to Rowell, is particularly true for animals, such as the harvest mouse, which are not normally social, and are not thought to interact much in the wild. While this could be the case for the interactions observed in this study, my anecdotal observations of the successful male apparently responding to some cue (possibly vocal) that the female and the non-successful male were interacting may suggest that some sort of asymmetrical interaction exists nonetheless between males and may occur in natural conditions.

Although it is not known for how long a male will consort closely with a female and be in position to chase away potential rivals, my observations suggest that harvest mice show at least rudimentary mate guarding, or courtship interference (Wong & Candolin 2005).  In all cases reported here, females mated with only one of the two males, which may have been a choice imposed by the males. It is possible that in a less confined environment the female would mate with more than one male.

### *3.4.4. Role of female-male proximity*

Overall the female spent more time in proximity to the successful, than the non-successful, male. In three of the four trials the successful male was largely responsible for maintaining his proximity to the female. This situation differs from other rodent species, such as rats (*Rattus norvegicus and Rattus rattus* - Erskine

1989), tassel-eared squirrels *Sciurus aberti* (Farentinos 1980) or common yellow-toothed cavy *Galea musteloides* (Sachser et al. 1999) in which the female approaches the male and actively solicits mating. In all these species females are promiscuous and – unlike in the harvest mouse - groups of males follow oestrous females.

Females spent more time in proximity to the non-successful male in the first hour of the trial in three of the four trials. This later changed, and the female spent more time in proximity to the successful male. There is evidence to suggest that the male which was eventually successful achieved this by his greater persistence in making efforts to be close to her, as suggested by the proximity indices between them, and by preventing the other male access to the female.

Perhaps by consistently chasing away the non-successful male (either during an interaction with the female [thus shortening their time together], or generally keeping him "out of the way" [and thus unavailable to the female]) on the one hand, and maintaining proximity to the female on the other, the successful male was actively responsible for the female spending more time with him than with the other male. In other words, the successful male's success seems to be a result of the male's actions, rather than of female choice. This contradicts the assumption made in the introduction, that mate choice is largely down to the female, and highlights a need to re-examine the prevalent idea (P. Rudd, Chester Zoo, pers. comm.; Trout, 1978b) that female harvest mice are dominant to males.

### *3.4.5. General conclusions*

There may not have been sufficient difference between paired males in basic biological measures to determine which of them mated with the female. It may be however that where there is greater asymmetry in these factors, that they do play a role. In Chapter 2 I found that female harvest mice preferred to mate with familiar males. However here where females were offered a choice of familiar and unfamiliar males, their mate choice seemed to be determined by male dominance as measured by winning interactions with the other male. Measures of proximity between the female and each of the males suggested that despite a tendency towards the beginning of trials for females to spend more time near males with which they did not eventually mate, overall they spent more time with their preferred mate. Proximity indices suggest that this may have been largely due to persistence on the part of the successful male securing him access to the female.

The results presented here do not provide conclusive support for the use (describe in Chapter 2) of time spent in proximity as a reliable proxy for sexual preference, but nor do they refute the suggestion that it is.

It may be that dominance is one of the traits upon which the female bases her choice of mate, as is common in other species (Horne & Ylonen 1996; Huck & Banks 1982; Rolland et al. 2003; Shapiro & Dewsbury 1986). If both familiarity and male dominance have a role in female mate choice then it may be that when these two factors are in conflict it is male dominance which determines the female's choice of mate, especially when the dominant male can directly influence that choice.

# Chapter 4. Discussion

## 4.1. Familiarity and dominance

The main objective of this study was to examine female mate choice in the harvest mouse. To this end the choice experiment, described in Chapter 2, examined the effect of familiarity on female mate choice. The results of this experiment showed that when females are in oestrus, and thus likely to be searching for a mate, they show preference for a familiar male over an unfamiliar one. This preference is stronger the larger the familiar male is compared with the unfamiliar one.

These experiments involved females interacting directly with males for only four hours. This time limit was necessary because the experimental setup involved the males being tethered. One alternative approach, that was considered but rejected, would have relied on size dimorphism to contain males in a separate chamber from which they were too large to exit, but which females could access (Rolland et al. 2003; Thom et al. 2004). However, harvest mice do not show size dimorphism (Harris & Trout 1991). No sexual interactions were observed during the duration of these trials.

In order to observe a more natural mating sequence, including copulations, a more naturalistic experiment was set up, described in Chapter 3, which – as none of the animals were restrained - allowed the recording of a full mating cycle. This had the added benefit that the males were completely unfettered and had full access to the female. A triadic design was devised to further investigate female mate choice and in an attempt to confirm whether using proximity as a proxy for mating preference is valid (this was not confirmed, nor refuted either). Such a design also provided information on interactions between the two males, which is of particular interest in light of the importance of resolving the effect of male competition on female mate choice as has been emphasised by Wong & Candolin (2005). The experiments revealed that the females invariably mated with the dominant male, regardless of whether he was the familiar male or not. A possible explanation of this could be that the males' dominance relations mask the females' preference, with the dominant male preventing mating between the female and the subdominant male. This possibility would be compatible with the observation that in some of the trials the female spent more time in proximity to the subdominant male in the first hour, but later spent much more time in proximity to the dominant. This change was most likely a result of the dominant male actively approaching her and staying in her vicinity, while also chasing the other male away.

Though it is the dominant male's actions which seem to determine his mating success in these experiments, rather than direct female choice, it is plausible that the female will gain at least some fitness benefits from mating with a dominant male. Wong & Candolin (2005) in their review of the literature could offer only two examples where dominant males override female preference for non dominant males – in the brown trout *Salmo trutta* (citing Petersson et al. 1999), and possibly in water striders *Aquarius remigis* (citing Sih et al. 2002), where the smaller energetic costs associated with mating with smaller males makes female preference for them likely. However, they go on to review more common cases in the literature in which females are able to mate with their preferred male despite harassment from more dominant males. These cases suggest that ultimately females mate with males that confer the highest fitness benefits. Therefore the observed matings in the triadic experiments are likely to be - at least partially - the result of female mate choice.

Dominance then may be amongst the male traits for which female harvest mice select. Indeed, females of many species prefer to mate with dominant males (Hoffmeyer 1982; Horne & Ylonen 1996; McElligott et al. 2001; West & Packer 2002; Wolff 1985) and will exhibit this preference even when free from male coercion (Drickamer 1992; Huck & Banks 1982; Rolland et al. 2003; Shapiro & Dewsbury 1986). The benefits to a female (and to her offspring) of mating with a dominant male may include less harassment from other males, access to better territories (though this is possibly not relevant in the harvest mouse) and good genes (Andersson 1994; Clutton-Brock & McAuliffe 2009).

An interesting possibility is suggested by the apparent courtship interference observed in the triadic experiment described in Chapter 3, when the dominant male responded to the female interacting with the other male by chasing the latter away. His behaviour was possibly in response to some form of vocalization emitted either by the female or the other male. This may be a form of ‘indirect mate choice’ (as suggested by Wiley & Poston 1996), where females influence which male they mate with by manipulating the males’ behaviour.

Cox & Le Boeuf (1977) first proposed that the female’s copulation calls in the elephant seal *Mirounga angustirostris* promote male aggressive competition for access to the female. The result, as the authors report, is that usually only the mature, high ranking males are able to achieve ejaculatory mounts. They suggest that this is the simplest strategy for a female to mate with the best male in the harem. The authors went on to propose that similar mechanisms, whereby females incite male competition, are to be expected in other polygynous species. A similar mechanism is possible in African elephants *Loxodonta africana* (Poole et al. 1988), where the authors suggest that the females’ post-copulatory sequence calls serve as a way to advertise the female’s sexual state. As oestrous females prefer high ranking musth males, the calls may serve to attract the few – and possibly distant – such males in the population. Semple (1998) suggested a similar mechanism could operate in the Barbary macaque *Macaca sylvanus*, and showed that advertising copulation, by way of distinctive copulation calls, afforded an indirect mate choice mechanism by which female Barbary macaques increase their chances of mating with higher ranking males.

Similarly, in birds Montgomerie & Thornhill (1989) - based on the pattern of female calls in the red jungle fowl and Lapland longspurs - proposed a general hypothesis - that calls given by female birds before and during egg laying advertise the female’s fertility and therefore incite intra-male competition. And Pizzari (2001) showed that distress calls uttered by female feral fowl *Gallus gallus domesticus* during copulation with low-ranking males increased the likelihood that they would be disrupted – and the females inseminated - by higher-raking males.

Other forms of advertising the female's fertility may serve a similar function of manipulating male behaviour. Some female fish for example, use visual cues to advertise their reproductive state, and this too has been suggested as a means to incite male competition which increases the female's chance to mate with the highest quality male (Benson 2007; Farr & Travis 1986). In the golden Syrian hamster *Mesocricetus auratus* vaginal secretions which attract males to the female, also increase intra-male aggressiveness (Fischer & Brown 1993).

In the case of harvest mice, the interactions between the female and the subdominant male, which were interrupted by the dominant male, as described in Chapter 3, did not involve any obvious sexual behaviour. There was no mounting, lordosis or even examination of the ano-genital region, but mostly consisted of mutual sniffing. Therefore it would seem inappropriate to categorise possible vocalizations that occurred during these encounters as 'copulation calls'. However, it is possible that a similar mechanism of indirect mate choice occurs in the harvest mouse through another type of vocalization, or a different cue altogether. Though not much is known about vocalization in adult harvest mice, there are indications that vocalizations are used during courtship (Trout 1978b), and other rodents are known to emit ultrasonic vocalizations (USVs) during nonaggressive encounters as well. For example, laboratory rats and mice produce vocalizations specific to nonaggressive interactions (Portfors 2007); prairie voles *Microtus ochrogaster* emit different rate of USVs depending on whether the individual they interact with is familiar to them or not (Lepri et al. 1988); and oestrous female golden hamsters *M.*

*auratus* emit higher rates of USVs than non-oestrous females before and after exposure to males (Floody 1979). A female's vocalizations while interacting with a male harvest mouse might therefore draw the attention of more dominant individuals, which chase the first male away, resulting in her mating with the most dominant male in the vicinity.

It is likely therefore that female harvest mice have a preference both for familiar males and for dominant ones, which is in accord with my finding that females showed greater preference for familiar males the larger they were compared to the unfamiliar one (Chapter 2). As is discussed in Chapter 2, one reason for a preference for familiar males is that familiarity with the male may be the result of higher marking rates which are associated with dominance (Brown & Macdonald 1985; Cheetham et al. 2008; Ralls 1971). In other words, familiarity can also be a proxy for dominance. And though in the study's triadic encounters male dominance seemed to have taken precedence over familiarity, it is possible that this particular interaction between the two traits is an artefact of the enclosed arena, which allowed the dominant male to exclude the other male more completely than might be possible in the wild.

## 4.2. Suggested further research

Given the above discussion, a natural direction for further study of mating behaviour in harvest mice is to observe it in more naturalistic environments. A better understanding about the interaction between familiarity and dominance in female mate choice would be obtained by repeating the scenario of a female with two males, one familiar and one unfamiliar, in a semi-natural enclosure. Such an enclosure will allow more room for the female and subdominant male to avoid the dominant male, and make it clearer whether the female chooses to mate with only the dominant male, or is forced to by her inability to avoid him.

Another fruitful avenue for future research would be a genetic study to find microsatellite loci appropriate for testing paternity. This could facilitate research to determine paternity of litters conceived in natural – or semi-natural – populations, and reveal the prevalence of mixed paternity litters. If multi male mating is common this would answer definitively the question of whether male dominance provides exclusive access to female harvest mice in the wild.

A different, but promising, research direction would be to study vocalization and its connection to mating, for example by using a playback experiment. Here a recording of a female interacting with a subdominant male would be played to a dominant male. This method may thus establish whether the dominant male indeed responds to such vocalizations, and whether chasing away a subdominant male is within the natural behavioural repertoire of male harvest mice, rather than an anecdotal occurrence.

## 4.3. Application to natural populations of harvest mice

When I started this study one of my motives was to provide – through better understanding of mate choice – some insight into social organisation in natural populations. This in turn would allow better predictions on subjects that are still poorly understood, such as dispersal and home range (Trout 1978b; Harris & Trout 1991). I believe my results offer important clues to the harvest mouse's natural history in ways that could not have been achieved in the wild. Indeed, my results are arguably one of the most detailed behaviour studies into mating interactions of this poorly known species that have ever been presented. This is important insofar as the harvest mouse is increasingly a species of conservation concern.

The anecdotal evidence that females will delay breeding when only their brothers are available (P. Rudd, Chester Zoo, pers. comm.) suggests that some form of inbreeding avoidance operates. Combined with my findings that females prefer familiar males, I posit that in the wild familiar males are not close kin. As males familiar to a female are probably from neighbouring or overlapping home-ranges, it is likely that neighbouring animals are usually not close kin. Such a spatial organisation can come about either by wide dispersal of both sexes, or by one sex being philopatric and the other one dispersing (Greenwood 1980). Previous findings suggest that harvest mice can move relatively long distances (Trout 1976, Trout 1978a, P. Rudd, Chester Zoo, pers. comm.), and there is some evidence to suggest that males travel further than females (Trout 1976), though it is unknown whether these movements are of dispersing animals. However the possibility that males move farther than females is compatible with dispersal patterns in the majority of mammal species, in which males disperse more than females (Greenwood 1980), and more specifically in rodents, for example in the grey-tailed voles *Microtus canicaudus* (Wolff et al. 1997) and in the wood mouse *Apodemus sylvaticus* (Kikkawa 1964). The preference for familiar males also supports the currently held assumption that harvest mice hold relatively stable home ranges, as familiarity requires repeated exposure to an individual's scent. However, as knowledge of the behavioural ecology of this species in the wild is so fragmentary, the spatial organisation of this species is still unclear.

The possibility that the dominant male responds to vocalizations – or some other cue - when the female interacts with other males, and that this behaviour is not restricted to immediately before or after mating, suggests that female and male (or at least dominant males) home ranges regularly overlap. This also raises the possibility that dominant males might exclude other males from female home ranges during mating season. Previously, only females excluding other females from the vicinity of their nesting area has been suggested (Trout 1978a).

One caveat to my interpretations is that caution is required when extrapolating from studies in captivity to behaviour in the wild (Wolff 2003). The two major experiments I conducted, as reported in this thesis, illustrate how carefully controlled choice experiments allow researchers to isolate one factor (familiarity), but offer only limited predictions about how other confounding factors (dominance) will affect behaviour in a natural population. Clearly, results of the sort I report can inform field studies and, hopefully, be validated by them. And while field based experiments are necessary to provide a comprehensive understanding of a species' natural behaviour, without the observations in captivity, the hypotheses and indicative results would not have been available for testing.

# Appendix - The Oestrous Cycle in the Harvest Mouse

## A1. Introduction

The oestrous cycle in rodents has been studied extensively, mainly in laboratory rats *Rattus norvegicus* and mice *Mus musculus*. Both have an oestrous cycle showing four stages - pro-oestrus, oestrus, metoestrus and dioestrus, and lasting 4-5 days (rats - Hubscher et al 2005, Freeman 2006, Schneider & Popik 2007; mice – Nelson et al. 1982, Rugh 1990). The same four stages and similar length of cycles are found in other Muridae spescies [for example in *R. rattus* and *Meriones tristrami* (Dewsbury et al. 1977) or in hairy-footed gerbils, genus *Gerbillurus*, though length of cycles is a little longer, 6-7 days (Dempster & Perrin 1989)].

The most common technique of monitoring the female oestrous cycle is by taking a vaginal smear (the scraping or swabbing of the vaginal walls) and assessing vaginal cytology which reflects the stage in the oestrous cycle the female is in at the time (Brown 1985; Freeman 2006; Gubernick & Addington 1994; Hubscher et al. 2005; Lefevre & McClintock 1988; Schneider & Popik 2007; Shapiro & Dewsbury 1986).

Currently no data are available about vaginal cytology or the oestrous cycle in the harvest mouse, therefore below I present data which have been collected during the experiments described in Chapters 2 and 3 of this thesis.

## A1.2. Methods

The harvest mice used were bred in Oxford University, using stock obtained from Chester Zoo (United Kingdom) and Wildwood Discovery Park (Kent, United Kingdom) and were bred with genetically distant individuals as far as possible, in order to keep a healthy, outbred, population. Animals were separated from opposite sex siblings before sexual maturity and from same sex siblings shortly thereafter. Animals were housed in individual tanks (Ferplast Geo Extra Large 11L 35X23X22 cm) until participation in an experiment.

The animal house was kept in a daily photoperiod of 16h light and 8h darkness and temperature was kept at a constant 21 degrees Celsius. These conditions simulate an English summer, the breeding season for the harvest mouse in Britain (June-September, Trout 1978a).

Vaginal secretions were collected using a sterile plastic pipette filled with saline (Goldman et al. 2007) and stained with 0.05% methylene blue. Slides were examined immediately, and were assessed for percentage and type of cells present as described, for laboratory rats, in LeFevre & McClintock (1988). Smears were defined as either leukocytic, if more than 20% of the cells present were leukocytes; or epithelial if more than 80% of the cells present were nucleated or cornified epithelial cells. A leukocytic smear (Figure A1(a)) was considered as indicating the metoestrous or dioestrous phases. An epithelial smear (Figure A1(b)) was considered as indicating oestrus or proestrus. However, a pro-oestrous smear in both lab rats (Freeman 2006) and mice (Rugh 1990) typically contain mainly nucleated epithelial cells, and most of the epithelial smears obtained from the harvest mice contained either mostly cornified cells or a mixture of cornified and nucleated cells. Therefore all epithelial smears were treated as indicating oestrus. An erroneous identification in this case would presumably have had only a minimal effect on the females’ behaviour as animals can also mate during vaginal pro-oestrus (Rugh 1990).

48 series of smears were taken, lasting between 2 and 11 days (mean – 3.5 ±0.3 days), from a total of 24 different females. The females’ age at the first day of a smear series ranged between 71 and 225 days (mean – 118.7 ±4.5 days).

Some females had previous encounters with males in previous experiments, but none of the females mated. All females had soiled bedding from a male’s cage added to their own for a minimum of 3 days before the first smear was taken (to familiarize the female with that male’s scent. See chapter 2 for details).

## A1.3. Results

No regular daily smears were taken beyond what was needed for the experiments described in this thesis, therefore smears were not collected for long enough to reach any conclusion regarding whether a regular oestrous cycle occurs and how long it lasts. However, as no data have been published about the oestrous cycle in the harvest mouse, I believe the following information is of value for future studies into the mating behaviour and physiology of this species.

Most females showed variation in their vaginal cytology, with the smears obtained switching between leukocytic and epithelial type smears, and vice versa.

However, out of the 24 females sampled, two females were never detected to be in oestrus:

1) One female's first smear, taken at the age of 133 days, showed leukocytes infiltrating a mostly epithelial smear, indicating she may have been in metoestrus at the time. For the following two days her smears showed growing proportion of leukocytes. The next series of smears from the same female (starting when she was 146 days) showed 11 consecutive days of leukocytic smears, with hardly any epithelial cells in them.
2) The second female had three consecutive days of leukocyte-only smears at the age of 71 days. The following series (starting when she was 85 days) had four consecutive days of leukocyte-only smears followed by three days with infiltration of round nucleated epithelial cells, which could indicate a development towards pro-oestrus.

Several females showed a prolonged period of leukocytic smears, with the longest being for 11 days (female 1, described above). Table A1 lists these occasions and the age of the female on the day of the first smear.

Though irregular oestrous cycle and persistent dioestrus can be related to the aging process (Lefevre & McClintock 1988), I could detect no obvious effect of age on whether or not a female went into oestrus, as the age of the females who showed a persistent tendency towards leukocytic smears was around the overall mean (118 days).

Table A1. Prolonged periods of leukocytic smears and the age of the female at their commencement.

| **Description of smears obtained** | **Age (in days) at first smear** |
|---|---|
| 11 consecutive days of leukocytic smears | 146 |
| 8 consecutive days of leukocytic smears, following two days of epithelial smears | 100 |
| 7 days of consecutive leukocytic smears | 100 |
| 6 days of consecutive leukocytic smears followed by two days of epithelial smears | 111 |
| 6 days of consecutive leukocytic smears followed by one day of an epithelial smear | 157 |

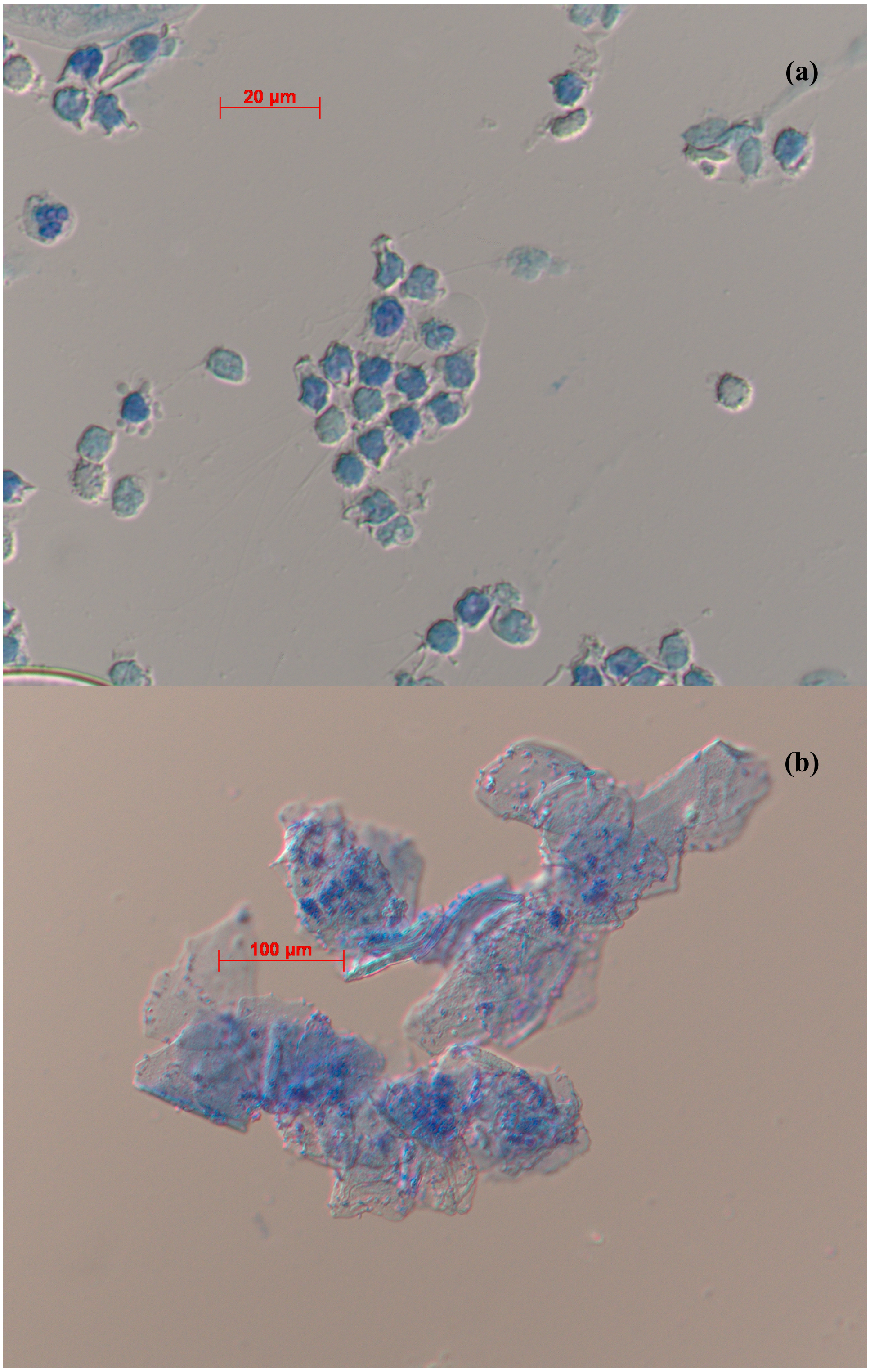

Figure A1. Photos of smear slides stained with 0.05% methylene blue – (a) leukocytes; (b) a cluster of epithelial cells. Images were taken using microscope software AxioVision 4.1.

# References

Andersson, M. B. 1994. *Sexual Selection*. Princeton: Princeton University Press.

Anthony, L. L. & Blumstein, D. T. 2000. Integrating behaviour into wildlife conservation: the multiple ways that behaviour can reduce N-e. *Biological Conservation*, 95, 303-315.

Bartlett, M. S. 1954. A note on the multiplying factors for various chi-2 approximations. *Journal of the Royal Statistical Society Series B-Statistical Methodology*, 16, 296-298.

Beacham, J. L. 1988. The relative importance of body size and aggressive experience as determinants of dominance in pumpkinseed sunfish, *Lepomis-gibbosus*. *Animal Behaviour*, 36, 621-623.

Berdoy, M. & Macdonald, D. W. 2005. Multiple male mating in brown rats, *Rattus norvegicus*: male coercion or female promiscuity. In: *The Second WildCRU Review* (Ed. by D. W. Macdonald, D. Randall & C. Hurst): Wildlife Conservation Research Unit, University of Oxford.

Berdoy, M., Smith, P. & MacDonald, D. W. 1995. Stability of social status in wild rats: age and the role of settled dominance. *Behaviour*, 132, 193-212.

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.

Bessa-Gomes, C., Danek-Gontard, M., Cassey, P., Moller, A. P., Legendre, S. & Clobert, J. 2003. Mating behaviour influences extinction risk: insights from demographic modelling and comparative analysis of avian extinction risk. *Annales Zoologici Fennici*, 40, 231-245.

Borries, C., Sommer, V. & Srivastava, A. 1991. Dominance, age, and reproductive success in free-ranging female hanuman langurs (*Presbytis-entellus*). *International Journal of Primatology*, 12, 231-257.

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

Brooks, R. J. & Schwarzkopf, L. 1983. Factors affecting incidence of infanticide and discrimination of related and unrelated neonates in male *Mus musculus*. *Behavioral and Neural Biology*, 37, 149-161.

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

Brown, R. E. 1985. The rodents I: effects of odours on reproductive physiology (primer effects). In: *Social Odours in Mammals* (Ed. by R. E. Brown & D. W. Macdonald), pp. 245-344. Oxford: Clarendon Press.

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

Buchanan, K. L. & Catchpole, C. K. 1997. Female choice in the sedge warbler, *Acrocephalus schoenobaenus*: multiple cues from song and territory quality. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 264, 521-526.

Carr, W. J., Kimmel, K. R., Anthony, S. L. & Schlocker, D. E. 1982. Female rats prefer to mate with dominant rather than subordinate males. *Bulletin of the Psychonomic Society*, 20, 89-91.

Charlton, B. D., Reby, D. & McComb, K. 2007. Female red deer prefer the roars of larger males. *Biology Letters*, 3, 382-385.

Cheetham, S. A., Thom, M. D., Beynon, R. J. & Hurst, J. L. 2008. The effect of familiarity on mate choice. *Chemical Signals in Vertebrates*, 11, 271-280.

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. & McAuliffe, K. 2009. Female mate choice in mammals. *Quarterly Review of Biology*, 84, 3-27.

Coopersmith, C. B. & Banks, E. M. 1983. Effects Of olfactory cues on sexual-behavior In the brown lemming, *Lemmus trimucronatus*. *Journal of Comparative Psychology*, 97, 120-126.

Côté, I. M. 2003. Knowledge of reproductive behavior contributes to conservation programs. In: *Animal Behavior and Wildlife Conservation* (Ed. by M. Festa-Bianchet & M. Apollonio), pp. 77-92. Washington: Island Press.

Cox, C. R. & Le Boeuf, B. J. 1977. Female incitation of male competition - mechanism in sexual selection. *American Naturalist*, 111, 317-335.

Cross, R. M. 1967. Studies on the ecology, physiology and taxonomy of the Harvest mouse, *Micromys minutus* (Pallas). Unpublished Ph.D. thesis, University of Southampton.

Dale, S. & Slagsvold, T. 1990. Random settlement of female pied flycatchers, *Ficedula hypoleuca* - significance of male territory size. *Animal Behaviour*, 39, 231-243.

Daly, M. 1977. Some experimental tests of the functional significance of scent-marking by gerbils (*Meriones unguiculatus*). *Journal of Comparative and Physiological Psychology*, 91, 1082-1094.

Dempster, E. R. & Perrin, M. R. 1989. The estrous-cycle and induction of estrous behavior in 4 species of hairy-footed gerbils (genus *Gerbillurus*). *Journal of Mammalogy*, 70, 809-811.

Derix, R., Hooff, J. V., Vries, H. D. & Wensing, J. 1993. Male and female mating competition in wolves: female suppression vs. male intervention. *Behaviour*, 127, 141-174.

Dewsbury, D. A. 1967. A quantitative description of the behavior of rats during copulation. *Behaviour*, 29, 156-178.

Dewsbury, D. A. 1979. Copulatory behavior of deer mice (*Peromyscus maniculatus*): II. A study of some factors regulating the fine structure of behavior. *Journal of Comparative and Physiological Psychology*, 93, 161-177.

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

Dewsbury, D. A., Estep, D. Q. & Lanier, D. L. 1977. Estrous cycles of 9 species of muroid rodents. *Journal of Mammalogy*, 58, 89-92.

Dickman, C. R. 1986. Habitat utilization and diet of the Harvest mouse, *Micromys minutus*, in an urban-environment. *Acta Theriologica*, 31, 249-256.

Dobson, A. P. & Lyles, A. M. 1989. The population-dynamics and conservation of primate populations. *Conservation Biology*, 3, 362-380.

Dobson, A. P. & Poole, J. H. 1998. Conspecific aggregation and conservation biology. In: *Behavioral Ecology and Conservation Biology* (Ed. by T. Caro). Oxford: Oxford University Press.

Docherty, S. J. 2009. Scent mark communication and competitive behaviour of the harvest mouse (*Micromys minutus*) Unpublished MPhil thesis, University of Liverpool.

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

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

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

Engel, C. R. 1990. Scent marking, residency, and female choice in the european rabbit (*Oryctolagus-cuniculus*). In: *Chemical Signals in Vertebrates 5* (Ed. by D. W. Macdonald, D. Mullerschwarze & S. E. Natynczuk), pp. 329-335. Oxford: Oxford University Press.

Erskine, M. S. 1989. Solicitation behavior in the estrous female rat: A review. *Hormones and Behavior*, 23, 473-502.

Erskine, M. S., Barfield, R. J. & Goldman, B. D. 1978. Intraspecific fighting during late pregnancy and lactation in rats and effects of litter removal. *Behavioral Biology*, 23, 206-218.

Fano, E., Martin, J. R. S. & Brain, P. F. 1997. Ethologically-determined responses of male mice in new dyadic encounters reflect their previous 'social status'. *Psicothema*, 9, 391-397.

Farentinos, R. C. 1980. Sexual solicitation of subordinate males by female tassel-eared squirrels (*Sciurus aberti*). *Journal of Mammalogy*, 61, 337-341.

Fischer, R. B. & Brown, P. S. 1993. Vaginal secretions increase the likelihood of intermale aggression in Syrian hamsters. *Physiology & Behavior*, 54, 213-214.

Fisher, H. S., Swaisgood, R. R. & Fitch-Snyder, H. 2003. Odor familiarity and female preferences for males in a threatened primate, the pygmy loris *Nycticebus pygmaeus*: applications for genetic management of small populations. *Naturwissenschaften*, 90, 509-512.

Floody, O. R. 1979. Behavioral and physiological analyses of ultrasound production by female hamsters (*Mesocricetus auratus*). *American Zoologist*, 19, 443-455.

Frank, F. 1957. Zucht und Gefangenschafts-Biologie der Zwergmaus (*Micromis minutus subobscurus* Fritsche). *Zeitschrift Fur Saugetierkunde-International Journal of Mammalian Biology*, 22, 1-44.

Freeman, M. E. 2006. Neuroendocrine control of the ovarian cycle of the rat. In: *Knobil and Neill's physiology of reproduction* (Ed. by J. D. Neill), pp. 2327- 2388: Academic Press.

Frynta, D., Volfova, R., Frankova-Novakova, M. & Stejskal, V. 2010. Oestrous females investigate the unfamiliar male more than the familiar male in both commensal and non-commensal populations of house mice. *Behavioural Processes*, 83, 54-60.

Giordano, A. L., Siegel, H. I. & Rosenblatt, J. S. 1984. Effects of mother-litter separation and reunion on maternal aggression and pup mortality in lactating hamsters. *Physiology & Behavior*, 33, 903-906.

Goldman, J. M., Murr, A. S. & Cooper, R. L. 2007. The rodent estrous cycle: characterization of vaginal cytology and its utility in toxicological studies. *Birth Defects Research Part B: Developmental and Reproductive Toxicology*, 80, 84-97.

Gosling, L. M. & Roberts, S. C. 2001. Scent-marking by male mammals: cheat-proof signals to competitors and mates. In: *Advances in the Study of Behavior, Vol 30*, pp. 169-217. San Diego: Academic Press Inc.

Grahn, M., Langefors, A. & Schantz, T. v. 1998. The importance of mate choice in improving viability in captive populations. In: *Behavioural Ecology and Conservation Biology* (Ed. by T. Caro). Oxford: Oxford University Press.

Grant, E. C. & Mackintosh, J. H. 1963. A comparison of the social postures of some common laboratory rodents. *Behaviour*, 21, 246-259.

Green, D. J. & Krebs, E. A. 1995. Courtship feeding in ospreys *Pandion haliaetus*: a criterion for mate assessment? *Ibis*, 137, 35-43.

Greene, C., Umbanhowar, J., Mangel, M. & Caro, T. 1998. Animal breeding systems, hunter selectivity, and consumptive use in wildlife conservation. *Behavioral Ecology and Conservation Biology*, 271-305.

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

Greig-Smith, P. W. 1982. Song-rates and parental care by individual male stonechats (*Saxicola torquata*). *Animal Behaviour*, 30, 245-252.

Gubernick, D. J. & Addington, R. L. 1994. The stability of female social and mating preferences in the monogamous California mouse, *Peromyscus californicus*. *Animal Behaviour*, 47, 559-567.

Hamilton, W. D. & Zuk, M. 1982. Heritable true fitness and bright birds - a role for parasites. *Science*, 218, 384-387.

Harris, S. & Trout, R. C. 1991. Harvest mouse *Micromys minutus*. In: *The Handbook of British Mammals* (Ed. by G. B. Corbet & S. Harris), pp. 233-239. Oxford: Blackwell Scientific.

Helfenstein, F., Wagner, R. H., Danchin, E. & Rossi, J. M. 2003. Functions of courtship feeding in black-legged kittiwakes: natural and sexual selection. *Animal Behaviour*, 65, 1027-1033.

Hepper, P. G. 1986. Kin recognition: Functions and mechanisms a review. *Biological Reviews*, 61, 63-93.

Hewitt, S. E., Macdonald, D. W. & Dugdale, H. L. 2009. Context-dependent linear dominance hierarchies in social groups of European badgers, *Meles meles*. *Animal Behaviour*, 77, 161-169.

Hiebert, S. M., Stoddard, P. K. & Arcese, P. 1989. Repertoire size, territory acquisition and reproductive success in the song sparrow. *Animal Behaviour*, 37, 266-273.

Hinde, R. & Atkinson, S. 1970. Assessing roles of social partners in maintaining mutual proximity, as exemplified by mother-infant relations in rhesus monkeys. *Animal Behaviour*, 18, 169.

Hodgdon, H. E. & Lancia, R. A. 1983. Behavior of the North American beaver, *Castor canadensis*. *Acta Zoologica Fennica*, 174, 99-103.

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.

Hohoff, C., Franzen, K. & Sachser, N. 2003. Female choice in a promiscuous wild guinea pig, the yellow-toothed cavy (*Galea musteloides*). *Behavioral Ecology and Sociobiology*, 53, 341-349.

Horn, J. L. 1965. A rationale and test for the number of factors in factor analysis. *Psychometrika*, 30, 179-185.

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

Hosken, D. J. & Stockley, P. 2003. Benefits of polyandry: a life history perspective. In: *Evolutionary Biology, Vol 33* (Ed. by R. J. MacIntyre & M. T. Clegg), pp. 173-194. New York: Kluwer Academic Publishing

Houde, A. E. & Torio, A. J. 1992. Effect of parasitic infection on male color pattern and female choice in guppies. *Behavioral Ecology*, 3, 346-351.

Hubscher, C. H., Brooks, D. L. & Johnson, J. R. 2005. A quantitative method for assessing stages of the rat estrous cycle. *Biotechnic & Histochemistry*, 80, 79-87.

Huck, U. W. & Banks, E. M. 1982. Male-dominance status, female choice and mating success in the brown lemming, *Lemmus trimucronatus*. *Animal Behaviour*, 30, 665-675.

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 discrimination between related and unrelated young. *Animal Behaviour*, 30, 1158-1165.

Huck, U. W., Lisk, R. D., Allison, J. C. & Vandongen, C. G. 1986. Determinants of mating success in the golden-hamster (*Mesocricetus auratus*) - social-dominance and mating tactics under seminatural conditions. *Animal Behaviour*, 34, 971-989.

Huck, U. W., Lisk, R. D., Miller, K. S. & Bethel, A. 1988. Progesterone levels and socially-induced implantation failure and fetal resorption in golden-hamsters (*Mesocricetus auratus*). *Physiology & Behavior*, 44, 321-326.

Hull, E. M. & Dominguez, J. M. 2007. Sexual behavior in male rodents. *Hormones and Behavior*, 52, 45-55.

Huntingford, F. A. & Turner, A. K. 1987. *Animal Conflict*. London: Chapman and Hall.

Hurst, J. L. 1987. Behavioral variation in wild house mice *Mus domesticus* Rutty - a quantitative assessment of female social-organization. *Animal Behaviour*, 35, 1846-1857.

Ishiwaka, R. & Mori, T. 1999. Early development of climbing skills in harvest mice. *Animal Behaviour*, 58, 203-209.

Ishiwaka, R., Kinoshita, Y., Satou, H., Kakihara, H. & Masuda, Y. 2010. Overwintering in nests on the ground in the harvest mouse. *Landscape and Ecological Engineering*, 6, 335-342.

IUCN. 2010. IUCN Red List of Threatened Species. Version 2010.3. www.iucnredlist.org.

Jamieson, I. G. & Colgan, P. W. 1989. Eggs in the nests of males and their effect on mate choice in the 3-spined stickleback. *Animal Behaviour*, 38, 859-865.

Janson, C. H. 1984. Female choice and mating system of the brown capuchin monkey *Cebus apella* (Primates: Cebidae). *Zeitschrift Fur Tierpsychologie-Journal of Comparative Ethology*, 65, 177-200.

Jennions, M. D. & Petrie, M. 2000. Why do females mate multiply? A review of the genetic benefits. *Biological Reviews*, 75, 21-64.

Jones, C. G., Heck, W., Lewis, R. E., Mungroo, Y., Slade, G. & Cade, T. 1995. The restoration of the mauritius kestrel *Falco punctatus* population. *Ibis*, 137, S173-S180.

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

Kaiser, H. 1970. A second generation little jiffy. *Psychometrika*, 35, 401-415.

Kaiser, H. 1974. An index of factorial simplicity. *Psychometrika*, 39, 31-36.

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.

Kenagy, G. J. & Trombulak, S. C. 1986. Size and function of mammalian testes in relation to body size. *Journal of Mammalogy*, 67, 1-22.

Kikkawa, J. 1964. Movement, activity and distribution of the small rodents *Clethrionomys glareolus* and *Apodemus sylvaticus* in woodland. *Journal of Animal Ecology*, 33, 259-299.

Klint, T. & Enquist, M. 1981. Pair formation and reproductive output in domestic pigeons. *Behavioural Processes*, 6, 57-62.

Kodric-Brown, A. 1992. Male dominance can enhance mating success in guppies. *Animal Behaviour*, 44, 165-167.

Kranz, K. R. 1991. Monogamy in the dik-dik. *Applied Animal Behaviour Science*, 29, 87-105.

Langbein, J. & Puppe, B. 2004. Analysing dominance relationships by sociometric methods - a plea for a more standardised and precise approach in farm animals. *Applied Animal Behaviour Science*, 87, 293-315.

Laudenslager, M. L., Natvig, C., Mikulich-Gilbertson, S., Blevins, M., Corcoran, C., Pierre, P. & Bennett, A. 2010. Challenges to bonnet monkey (*Macaca radiata*) social groups: mother–infant dyad and infant social interactions. *Developmental Psychobiology*, 52, 465-474.

Lefevre, J. & McClintock, M. K. 1988. Reproductive senescence in female rats - a longitudinal-study of individual-differences in estrous cycles and behavior. *Biology of Reproduction*, 38, 780-789.

Legendre, S., Clobert, J., Moller, A. P. & Sorci, G. 1999. Demographic stochasticity and social mating system in the process of extinction of small populations: the case of passerines introduced to New Zealand. *American Naturalist*, 153, 449-463.

Lepri, J. J., Theodorides, M. & Wysocki, C. J. 1988. Ultrasonic vocalizations by adult prairie voles, *Microtus ochrogaster*. *Cellular and Molecular Life Sciences*, 44, 271-273.

Love, R. A., Webon, C., Glue, D. E. & Harris, S. 2000. Changes in the food of British barn owls (*Tyto alba*) between 1974 and 1997. *Mammal Review*, 30, 107-129.

Macdonald, D. W., Mace, G. & Rushton, S. 1998. *Proposals for future monitoring of British mammals*. London: Department of the Environment, Trasport and the Regions.

Macdonald, D. W., Newman, C., Stewart, P. D., Domingo-Roura, X. & Johnson, P. J. 2002. Density-dependent regulation of body mass and condition in badgers (*Meles meles*) from Wytham Woods. *Ecology*, 83, 2056-2061.

Maestripieri, D. 1992. Functional aspects of maternal aggression in mammals. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, 70, 1069-1077.

Mallory, F. F. & Brooks, R. J. 1978. Infanticide and other reproductive strategies in collared lemming, *Dicrostonyx groenlandicus*. *Nature*, 273, 144-146.

Martin, P. & Bateson, P. 1993. *Measuring Behaviour: an introductory guide*, Second edn. Cambridge: Cambridge University Press.

Mathews, F., Honess, P. E. & Wolfensohn, S. 2002. Use of inhalation anaesthesia for wild mammals in the field. *Veterinary Record*, 150, 785-787.

McElligott, A. G. & Hayden, T. J. 2001. Postcopulatory vocalizations of fallow bucks: who is listening? *Behavioral Ecology*, 12, 41-46.

McElligott, A. G., Gammell, M. P., Harty, H. C., Paini, D. R., Murphy, D. T., Walsh, J. T. & Hayden, T. J. 2001. Sexual size dimorphism in fallow deer (*Dama dama*): do larger, heavier males gain greater mating success? *Behavioral Ecology and Sociobiology*, 49, 266-272.

Meerlo, P., Overkamp, G. J. F., Benning, M. A., Koolhaas, J. M. & Van Den Hoofdakker, R. H. 1996. Long-term changes in open field behaviour following a single social defeat in rats can be reversed by sleep deprivation. *Physiology & Behavior*, 60, 115-119.

Møller, A. P. 1992. Parasites differentially increase the degree of fluctuating asymmetry in secondary sexual characters. *Journal of Evolutionary Biology*, 5, 691-699.

Møller, A. P. & Pomiankowski, A. 1993. Fluctuating asymmetry and sexual selection. *Genetica*, 89, 267-279.

Montgomerie, R. & Thornhill, R. 1989. Fertility advertisement in birds - a means of inciting male-male competition. *Ethology*, 81, 209-220.

Motro, U. 1991. Avoiding inbreeding and sibling competition - the evolution of sexual dimorphism for dispersal. *American Naturalist*, 137, 108-115.

Nelson, J. F., Felicio, L. S., Randall, P. K., Sims, C. & Finch, C. E. 1982. A longitudinal study of estrous cyclicity in aging C57BL/6J mice: I. Cycle frequency, length and vaginal cytology. *Biology of Reproduction*, 27, 327-339.

Oparin, M. & Oparina, O. 2009. The role of anthropogenic and natural factors in changes in mesophilic rodent distribution in steppes between the Ural and Volga Rivers. *Biology Bulletin*, 36, 380-387.

Özkan, B., Yiğit, N. & Çolak, E. 2003. A study on *Micromys minutus* (Pallas, 1771) (Mammalia: Rodentia) in Turkish Thrace. *Turkish Journal of Zoology*, 27, 55-60.

Parker, P. G. & Waite, T. A. 1997. Mating systems, effective population size, and conservation of natural populations. In: *Behavioral Approaches to Conservation in the Wild* (Ed. by J. R. Clemmons & R. Buchholz), p. 382 Cambridge Cambridge University Press.

Patris, B. & Baudoin, C. 1998. Female sexual preferences differ in *Mus spicilegus* and *Mus musculus domesticus*: the role of familiarization and sexual experience. *Animal Behaviour*, 56, 1465-1470.

Paul, L. 1986. Infanticide and maternal aggression - synchrony of male and female reproductive strategies in mice. *Aggressive Behavior*, 12, 1-11.

Penn, D. & Potts, W. K. 1998. Chemical signals and parasite-mediated sexual selection. *Trends in Ecology & Evolution*, 13, 391-396.

Perrow, M. & Jowitt, A. 1995. What future for the harvest mouse? *British Wildlife*, 6, 356-365.

Petersson, E., Jarvi, T., Olsen, H., Mayer, I. & Hedenskog, M. 1999. Male-male competition and female choice in brown trout. *Animal Behaviour*, 57, 777-783.

Pierce, J. D. & Dewsbury, D. A. 1991. Female preferences for unmated versus mated males in 2 species of voles (*Microtus ochrogaster* and *Microtus montanus*). *Journal of Comparative Psychology*, 105, 165-171.

Poole, J. H., Payne, K., Langbauer, W. R. & Moss, C. J. 1988. The social contexts of some very low-frequency calls of African elephants. *Behavioral Ecology and Sociobiology*, 22, 385-392.

Portfors, C. V. 2007. Types and functions of ultrasonic vocalizations in laboratory rats and mice. *Journal of the American Association for Laboratory Animal Science*, 46, 28-34.

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.

Pusey, A. & Wolf, M. 1996. Inbreeding avoidance in animals. *Trends in Ecology & Evolution*, 11, 201-206.

Pusey, A. E. 1987. Sex-biased dispersal and inbreeding avoidance in birds and mammals. *Trends in Ecology & Evolution*, 2, 295-299.

Quader, S. 2005. Mate choice and its implications for conservation and management. *Current Science*, 89, 1220-1229.

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

Ralls, K. 1971. Mammalian scent marking. *Science*, 171, 443-449.

Randall, J. A. 1991. Sandbathing to establish familiarity in the Merriams kangaroo rat, *Dipodomys merriami*. *Animal Behaviour*, 41, 267-275.

Randall, J. A., Hekkala, E. R., Cooper, L. D. & Barfield, J. 2002. Familiarity and flexible mating strategies of a solitary rodent, *Dipodomys ingens*. *Animal Behaviour*, 64, 11-21.

Ricankova, V., Sumbera, R. & Sedlacek, F. 2007. Familiarity and partner preferences in female common voles, *Microtus arvalis*. *Journal of Ethology*, 25, 95-98.

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

Roberts, S. C. & Gosling, L. M. 2003. Genetic similarity and quality interact in mate choice decisions by female mice. *Nature Genetics*, 35, 103-106.

Roberts, S. C. & Gosling, L. M. 2004. Manipulation of olfactory signaling and mate choice for conservation breeding: A case study of harvest mice. *Conservation Biology*, 18, 548-556.

Rolland, C., Macdonald, D. W., De Fraipont, M. & Berdoy, M. 2003. Free female choice in house mice: leaving best for last. *Behaviour*, 140, 1371-1388.

Rowe, F. P. 1958. Some observations on harvest mice from the corn ricks of a Hamshire farm. *Proceedings of the Zoological Society of London*, 131, 320-323.

Rowe, F. P. & Taylor, E. J. 1964. The number of harvest mice (*Micromys minutus*) in corn-ricks. *Proceedings of the Zoological Society of London*, 142, 181-185.

Rowell, T. E. 1974. The concept of social dominance. *Behavioral Biology*, 11, 131-154.

Rugh, R. 1990. *The Mouse: Its Reproduction and Development*: Oxford University Press.

Sachser, N., Schwarz-Weig, E., Keil, A. & Epplen, J. T. 1999. Behavioural strategies, testis size, and reproductive success in two caviomorph rodents with different mating systems. *Behaviour*, 136, 1203-1217.

Salo, A. L. & Dewsbury, D. A. 1995. 3 Experiments on mate choice in meadow voles (*Microtus pennsylvanicus*). *Journal of Comparative Psychology*, 109, 42-46.

Schleich, C. & Busch, C. 2002. Acoustic signals of a solitary subterranean rodent *Ctenomys talarum* (Rodentia: Ctenomyidae): physical characteristics and behavioural correlates. *Journal of Ethology*, 20, 123-131.

Schneider, T. & Popik, P. 2007. Attenuation of estrous cycle-dependent marble burying in female rats by acute treatment with progesterone and antidepressants. *Psychoneuroendocrinology*, 32, 651-659.

Schüler, L. & Renne, U. 1988. Relationship of social rank in mice to growth, endurance, and fertility. *Aggressive Behavior*, 14, 205-211.

Schulte, B. A. & Rasmussen, L. E. L. 1999. Signal-receiver interplay in the communication of male condition by Asian elephants. *Animal Behaviour*, 57, 1265-1274.

Semple, S. 1998. The function of Barbary macaque copulation calls. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 265, 287-291.

Setchell, J. M. & Dixson, A. F. 2001. Changes in the secondary sexual adornments of male mandrills (*Mandrillus sphinx*) are associated with gain and loss of alpha status. *Hormones and Behavior*, 39, 177-184.

Shapiro, L. E. & Dewsbury, D. A. 1986. Male-dominance, female choice and male copulatory-behavior In 2 species of voles (*Microtus ochrogaster* and *Microtus montanus*). *Behavioral Ecology and Sociobiology*, 18, 267-274.

Shapiro, L. E. & Dewsbury, D. A. 1990. Differences in affiliative behavior, pair bonding, and vaginal cytology in 2 species of vole (*Microtus-ochrogaster* and *Microtus-montanus*). *Journal of Comparative Psychology*, 104, 268-274.

Shapiro, L. E., Austin, D., Ward, S. E. & Dewsbury, D. A. 1986. Familiarity and female mate choice In 2 species of voles (*Microtus ochrogaster* and *Microtus montanus*). *Animal Behaviour*, 34, 90-97.

Shier, D. M. 2009. Behavioral ecology and translocation of the endangered Stephens' kangaroo rat (*Dipodomys stephensi*). pp. 1-28. Escondido: San Diego Zoo Institute for Conservation Research.

Sih, A., Lauer, M. & Krupa, J. J. 2002. Path analysis and the relative importance of male-female conflict, female choice and male-male competition in water striders. *Animal Behaviour*, 63, 1079-1089.

Sillero-Zubiri, C., Gottelli, D. & Macdonald, D. W. 1996. Male philopatry, extra pack copulations and inbreeding avoidance in Ethiopian wolves (*Canis simensis*). *Behavioral Ecology and Sociobiology*, 38, 331-340.

Sillero-Zubiri, C., Marino, J., Gottelli, D. & Macdonald, D. W. 2004. Afroalpine ecology, solitary foraging and intense sociality amongst Ethiopian wolves. In: *The Biology and Conservation of Wild Canids* (Ed. by D. W. Macdonald & C. Sillero-Zubiri), pp. 311-323. Oxford, UK: Oxford University Press.

Soule, M. E. 1982. Allomeric variation .1. the theory and some consequences. *American Naturalist*, 120, 751-764.

Soule, M. E. & Cuzinroudy, J. 1982. Allomeric variation .2. developmental instability of extreme phenotypes. *American Naturalist*, 120, 765-786.

Stockley, P. 2003. Female multiple mating behaviour, early reproductive failure and litter size variation in mammals. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 270, 271-278.

Sutherland, W. J. 1998. The importance of behavioural studies in conservation biology. *Animal Behaviour*, 56, 801-809.

Swaddle, J. P. & Cuthill, I. C. 1994. Female zebra finches prefer males with symmetric chest plumage. *Proceedings: Biological Sciences*, 258, 267-271.

Swaisgood, R. R., Lindburg, D. G., Zhou, X. P. & Owen, M. A. 2000. The effects of sex, reproductive condition and context on discrimination of conspecific odours by giant pandas. *Animal Behaviour*, 60, 227-237.

Tabachnick, B. G. & Fidell, L. S. 2007. *Using Multivariate Statistics*, 5th edn. Boston: Pearson Education.

Tai, F. D., Wang, T. Z. & Zhao, Y. J. 2000. Inbreeding avoidance and mate choice in the mandarin vole (*Microtus mandarinus*). *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, 78, 2119-2125.

Tang-Martinez, Z., Mueller, L. L. & Taylor, G. T. 1993. Individual odors and mating success in the golden-hamster, *Mesocricetus-auratus*. *Animal Behaviour*, 45, 1141-1151.

Thom, M. D., Macdonald, D. W., Mason, G. J., Pedersen, V. & Johnson, P. J. 2004. Female American mink, *Mustela vison*, mate multiply in a free- choice environment. *Animal Behaviour*, 67, 975-984.

Thompson, K. V., Roberts, M. & Rall, W. F. 1995. Factors affecting pair compatibility in captive kangaroo rats, *Dipodomys heermanni*. *Zoo Biology*, 14, 317-330.

Trail, P. W. 1985. Courtship disruption modifies mate choice in a lek-breeding bird. *Science*, 227, 778-780.

Trivers, R. L. 1972. Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man* (Ed. by B. Campbell), pp. 136–179. London: Heinemann.

Trout, R. C. 1976. An ecological study of populations of wild Harvest mice *(Micromys minutus soricinus* Hermann). Unpublished Ph.D. thesis, University of London.

Trout, R. C. 1978a. A review of studies on populations of wild harvest mice (*Micromys minutus* (Pallas). *Mammal Review*, 8, 143-158.

Trout, R. C. 1978b. A review of studies on captive harvest mice *(Micromys minutus* (Pallas)*)*. *Mammal Review*, 8, 159-175.

Turner, B. N. & Iverson, S. L. 1973. Annual cycle of aggression in male *Microtus pennsylvanicus*, and its relation to population parameters. *Ecology*, 54, 967-981.

Vahed , K. 1998. The function of nuptial feeding in insects: a review of empirical studies. *Biological Reviews*, 73, 43-78.

Watson, P. J. & Thornhill, R. 1994. Fluctuating asymmetry and sexual selection. *Trends in Ecology & Evolution*, 9, 21-25.

Wauters, L., Dhondt, A. A. & Devos, R. 1990. Factors affecting male mating success in red squirrels (*Sciurus vulgaris*). *Ethology Ecology & Evolution*, 2, 195-204.

Webster, B. A., Gartshore, G. R. & Brooks, R. J. 1981. Infanticide in the meadow vole, *Microtus pennsylvanicus*: significance in relation to social system and population cycling. *Behavioral and Neural Biology*, 31, 342-347.

West, P. M. & Packer, C. 2002. Sexual selection, temperature, and the lion's mane. *Science*, 297, 1339-1343.

White, P. J., Fischer, R. B. & Meunier, G. F. 1984. Discrimination of male social-status by female hamsters. *Psychological Reports*, 55, 487-492.

Wiggins, D. A. & Morris, R. D. 1986. Criteria for female choice of mates - courtship feeding and paternal care in the common tern. *American Naturalist*, 128, 126-129.

Wiley, R. H. & Poston, J. 1996. Perspective: indirect mate choice, competition for mates, and coevolution of the sexes. *Evolution*, 50, 1371-1381.

Wilson, E. O. 1975. *Sociobiology : The New Synthesis*. Cambridge, Mass.: Harvard University Press.

Wolff, J. O. 2003. Laboratory studies with rodents: facts or artifacts? *Bioscience*, 53, 421-427.

Wolff, J. O. & Cicirello, D. M. 1989. Field evidence for sexual selection and resource competition infanticide in white-footed mice. *Animal Behaviour*, 38, 637-642.

Wolff, J. O. & Dunlap, A. S. 2002. Multi-male mating, probability of conception, and litter size in the-prairie vole (*Microtus ochrogaster*). *Behavioural Processes*, 58, 105-110.

Wolff, J. O. & Macdonald, D. W. 2004. Promiscuous females protect their offspring. *Trends in Ecology & Evolution*, 19, 127-134.

Wolff, J. O., Schauber, E. M. & Edge, W. D. 1997. Effects of habitat loss and fragmentation on the behavior and demography of gray-tailed voles. *Conservation Biology*, 11, 945-956.

Wolff, R. J. 1985. Mating behavior 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.

Wong, B. B. M. & Candolin, U. 2005. How is female mate choice affected by male competition? *Biological Reviews*, 80, 559-571.

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

Ylonen, H., Koskela, E. & Mappes, T. 1997. Infanticide in the bank vole (*Clethrionomys glareolus*): occurrence and the effect of familiarity on female infanticide. *Annales Zoologici Fennici*, 34, 259-266.

Yoerg, S. I. 1999. Solitary is not asocial: effects of social contact in kangaroo rats (Heteromyidae : *Dipodomys heermanni*). *Ethology*, 105, 317-333.

Zahavi, A. 1975. Mate selection - selection for a handicap. *Journal of Theoretical Biology*, 53, 205-214.

Zenuto, R. R., Estavillo, C. & Fanjul, M. S. 2007. Familiarity and mating behavior in the subterranean rodent *Ctenomys talarum* (tuco-tuco). *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, 85, 944-955.

Zuk, M., Simmons, L. W. & Cupp, L. 1993. Calling characteristics of parasitized and unparasitized populations of the field cricket *Teleogryllus oceanicus*. *Behavioral Ecology and Sociobiology*, 33, 339-343.