CHEMICAL COMMUNICATION IN WILD NORWAY RATS

(*Rattus norvegicus* Berkenhout)

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ABSTRACT

This study examined the urine and faecal scent marking behaviour and investigatory responses of wild Norway rats (*Rattus norvegicus* Berkenhout) kept in large, semi-natural enclosures to assess the role these scents play in their communication system.

For the first time, this study has shown that Norway rats deposit faecal scent marks in response to odour cues and form latrines. The spatial distribution of faeces was highly uneven. Most faeces deposited in open areas were found in clusters occupying less than 1 m^2 which were termed latrines. Rats spent more time at feeders and in other areas which were almost devoid of faeces than at these latrines. This suggests that latrines were created deliberately, perhaps for communication.

Rats discriminated among faeces from different donors with respect to their investigation, presumably using olfactory cues. They faecal marked in response to urine cues from rats belonging to other colonies, although they did not faecal mark in response to their own urine cues or to a novel non-social stimulus (clean tiles). Investigation and faecal marking was aimed mainly towards urine from individuals of the marker's own sex. This suggests that faecal marking may play a role in communication between competitors.

Urine was deposited as discrete marks around the enclosures, in an uneven distribution. The highest density of marks was found by the enclosure walls and nest areas. Rats showed a greater urine marking response towards introduced clean surfaces than towards surfaces they had already marked, ensuring that their home area was always covered with their urine marks. Close monitoring of urine

marking on clean surfaces showed that male rats had a marking rate three times greater than that of females. This could not be attributed solely to weight differences between males and females.

Rats also urine marked in response to urine deposited by rats from other colonies. Urine from unfamiliar rats of the subject's own sex stimulated more investigation than urine from the opposite sex, though donors were immature. These results suggest that urine marking also plays a role in communication between competitors.

Testing individuals in their home enclosure, using scent marks deposited naturally by rats, and the contexts in which scent stimuli are deposited by donors (e.g. as part of their home range) and found by residents (e.g. finding intruder's home range marks in the resident's home range) were essential factors in determining their response to olfactory cues. The importance of these factors is discussed.

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CHAPTER ONE
INTRODUCTION.

Introductory note.

Throughout this thesis, the term 'rat' or 'wild rat' will be used as a synonym of Brown or Norway rat, *Rattus norvegicus*, Berkenhout. In chapters 3 to 5, the term 'residents' is used to refer to the rats belonging to the colony being tested, whereas the term 'non-residents' refers to unfamiliar or neighbour rats from colonies other than that being tested.

1.1. Introduction to scent marking.

The use of olfactory cues for a number of functions (orientation, sexual or competitive communication, individual recognition, etc.) is very widespread among vertebrates (Stoddart, 1980; section 1.3). Such exchange of information using chemicals, generally air-borne or diluted in water, is termed chemical communication (Agosta, 1992). Terrestrial mammals preferentially use air-borne chemicals and the type of chemical communication they use is termed olfactory communication (Thiessen and Rice, 1976). Although individuals may use chemicals released directly in the air, many animals, especially mammals, deposit their own olfactory cues as scent marks on the substratum for orientation or social communication (Alberts, 1992). Not all odoriferous substances deposited onto the substratum play a role in communication (e.g., urine and faeces, although odoriferous, may be voided purely as physiological excretions). However, very often odoriferous substances play a role in communication. I shall reserve

the term 'scent marking' for scents specifically deposited for communication or orientation.

When scent marking, individuals deposit chemical compounds onto the ground or onto objects in an animal's environment (Johnson, 1973), as well as onto conspecifics and themselves (Gosling, 1982) for communication or orientation.

1.1.1 Definition of communication.

Scent marking is one of several possible channels of communication. Communication is very important for animals because it mediates the interactions between them. Animals need information about the intentions or status of other animals. Broadcasters benefit by sending messages about themselves, irrespective of whether such information is true or not. According to Krebs and Davies (1993):

> Communication is the process in which actors use especially designed signals or displays to modify the behaviour of reactors.

Signals evolved because it was beneficial for a responding individual to read any cues about the intention of the individual producing the signal. Elimination products (urine, faeces, etc.) contain information regarding the internal state of the donor (a remarkable proportion of clinical biochemistry is based on diagnosing diseases using the chemical alterations they cause on these excreted products). Animals probably evolved the ability to detect information from these scents for their own benefit (Albone, 1984; Brown, 1995). Presumably, the donor then evolved the

behaviour of depositing scents in a manner, location and composition which maximised the chance of the scent being detected with the lowest energetic expenditure (Alberts, 1992), although costly chemical signals may have evolved to avoid cheating (see below). As Dawkins (1986) discusses in her review, it is particularly important to stress that signals have evolved especially to influence the behaviour of other conspecifics, and that animals may leave information (for instance body excretions) that influences the behaviour of conspecifics without the aim of communicating with them and that such information, consequently, does not constitute a signal.

The signaller usually benefits from the response triggered in the individual towards whom the message was aimed (although there is always a risk of incurring a cost if, for example, the signal is intercepted by a predator). The receiver reads or interprets a signal because he/she perceives the information the signal carries as beneficial to him/her. However, sometimes the signal is not honest and the response of the receiver can be detrimental to his/her fitness. The females of some species of fireflies of the genus *Photuris*, for example, use the female mating signals of another firefly species of the genus *Photinus*, to attract *Photinus* males and then eat them (Lloyd, 1975).

Krebs and Dawkins (1978) have suggested that manipulation by the sender lies at the heart of communication. However, Inglis and Shepherd (1990) have argued that this cannot be taken as the general rule because many signals (called honest signals) cannot be faked and, in other instances, the sender would not benefit from cheating. For example, a cheater of low competitive ability may obtain a short term advantage by mimicking signals from an

individual of higher competitive ability. However, in the long term, its greater vulnerability to attacks by truly dominant individuals may not offset the cost of cheating.

Smith (1968) pointed out that the message perceived by the receiver is not always that intended by the sender (see review in Inglis and Shepherd, 1990). For example, a signal intended to attract mates may attract predators too. They have suggested that the term 'message' should be reserved for the information that the signal has been selected to convey, whereas the term 'meaning' should be applied to the information the receiver reads. For example, the scent marks that male black rats deposit before a contest with the dominant male of a group (Ewer, 1971) whose message seems to be 'I defy you' may be read by a receptive female as 'I am a good mate'. However, signals may have multiple purposes and, therefore it may be difficult to know which ones were intended by the sender. Generally speaking, the difference between meaning and message makes sense in situations when the meaning for the receiver is either detrimental or neutral to the sender. For example, it seems difficult to be sure whether range marks are only intended to keep intruders out, attract mates, orientate the marking individual, or perhaps to establish its dominance over other individuals living in the area who do not deposit range marks (or for all these functions). It seems less likely that range marks have evolved because they signal food for a predator, or because they indicate the presence of the marking individual to a species which does not interact with it. In this case we can be reasonably sure that the meaning obtained was not that intended by the sender.

Another important idea reviewed in Inglis and Shepherd (1990) is that the meaning may vary with the context. For example,

if an object scent marked by an individual as part of its territory was introduced in another individual's territory, the signal would likely constitute a challenge for the resident.

1.1.2. Advantages of chemical over other means of communication.

Although communication can be established through any sensory channel, each channel of communication has some advantages and disadvantages over the others (Table 1.1 compares some of these characteristics for different sensory channels). These

Table 1.1 Properties of the major channels of communication (modified after Alcock, 1989).

	Sensory Channel			
Characteristics	Chemical	Acoustical	Visual	Tactile
Transmission range	Short to long	Long	Medium	Very short
Speed of transmission	Slow[1]	Fast	Fast	Fast
Can it skip barriers?	Yes	Yes	No	No[2]
Nocturnal use	Yes	Yes	No[3]	Yes
Fadeout time	Slow	Fast	Fast	Fast
Locatability of sender	Difficult/ fairly[4]	Fairly	Easy	Easy
Cost to send[5]	Low	High	Low to moderate	Low to moderate

advantages, along with the influence of environmental, energetic and other constraints, will make some sensory channels more

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[1]This does not necessarily mean that chemical signals take a long time to reach the receiver. Signals acting at short range, for instance, have to travel only a minimum space.
[2]Except tactile vibrations transmitted through water or ground.
[3]Except bioluminiscent signals.
[4] Some signals, like sex attractants, allow fairly easy locatability
[5]Some signals, such as those advertising status or mate quality, are deliberately expensive to avoid cheating.

suitable for some species, more suitable for certain situations within a species, or more suitable for broadcasting certain types of message.

For example, many mammals like rats are nocturnal and under such conditions it would be impractical to use visual displays for communication. Birds, on the other hand, spend a great proportion of their lives in the air, where visual signals are the cheapest method for communication provided that enough light is available. However, because of air currents, chemical signalling would be almost useless in this environment.

1.1.3. Basic concepts in chemical communication.

Traditionally, the chemical substances produced by one individual that trigger a response in another from the same or another species were termed *pheromones*. The word pheromone comes from two Greek words: *pherein*, to transfer, and *hormon*, to excite; and was first used by Martin Lüscher and Peter Karlson (Agosta, 1992). The classical work of Butenandt and other researchers (Hecker and Butenandt, 1984) on the sex attractant of the silk-worm moth, *Bombyx mori*, a chemical produced by females to attract males, created the idea in those not expert in the field, that a pheromone was a single chemical compound which triggered a fixed, particular response in a species.

In mammals, however, complex scents are generally used and the response they elicit is more flexible. Scents used by mammals in communication are usually formed from several chemicals (Gorman, 1990; Agosta, 1992). Often, some of the constituents do not appear to have biological importance on their own (Albone, 1984). This may happen because a subset of active compounds need the presence of each other to produce the desired effect. In addition,

mammals usually present a much more flexible response to chemical signals than insects, and such responses greatly depend on the context in which the scent is released and the social status of the receiver (Albone, 1984; Inglis and Shepherd, 1990).

In this case, the idea of a one compound-one response relationship fails and Albone (1984) has suggested the more appropriate idea of a chemical image. According to this idea, a mammal presents chemical images which differ from single-message pheromones in a similar way as an optical image differs from a single-message optical signal (e.g., a traffic light). These chemical images are differentiated with regard to:

-Chemical composition.

-Spatial distribution over the body of the sender and in the environment (the location where the scent is deposited).

-Time. This relates both to decay as high volatiles disperse, and to any change in composition of the scent due to decomposition (typically by bacteria) through time, also regarded as ageing of scents.

However, although different to pheromones in insects, signalling scents in mammals and other taxa still present similar modes of action and they can be regarded as *releasers* or *primers* (Thiessen and Rice, 1976):

-A releaser is a pheromone which triggers a more or less immediate response mainly through the nervous system.

-A primer pheromone, on the other hand, changes the physiology of an individual, usually over a long period of time. Many of these primer pheromones affect the reproductive capacity of individuals, either promoting or inhibiting it (Brown 1985a).

-Hurst (1993) has proposed that mammalian scents could prime (long lasting effect) the behaviour of conspecifics (e.g., reducing scent marking, increasing aggression, etc.) towards the marking individual.

1.2. Constraints on scent mark deposition.

The environment and the nature of the products used impose critical constraints on scent marking. Some of the factors affecting scent marking are: the rate of production of the scent, its fade out time, the carrier used (if it is water/lipid soluble, if it is released with faeces, etc.), size of the area needing to be marked and the amount of information it has to convey. These will be discussed in the next paragraphs.

Selection should act in a way that maximises transmission efficiency (Gorman, 1990; Alberts, 1992). Scent glands, for example, should interface with the substratum that the animal usually contacts, and their secretion should be readily available (Thiessen and Rice, 1976). The amount of product ready for deposition will impose a constraint on the mode and pattern of deposition. Urine, for example is more abundant and more energetically cheap to produce than secretions from sebaceous glands, and could be more easily used for marking the home range substratum than sebum. In this case, part of the reason for the additional costs of producing sebum may be the greater energy required to produce a lipid base compared with the cheap water base of urine.

Time also imposes constraints on scent marks. Olfactory cues should have low volatility to last long enough to be encountered by the individuals they are intended for, but they should also be

volatile enough to be perceived from an appropriate distance to be useful. Alberts (1992) found that sex attractants are the most volatile in order to attract mates during the period the sender is sexually active. Range marks, on the contrary, are less volatile, as these have to last long enough in the environment to be detected by intruders. Some odours may need to be non-volatile because the information they encode is aimed at those individuals contacting them, usually by means of the vomeronasal organ (Wysocki, 1979).

Despite the fact that heavier molecules are less volatile, and thus, they reach lower concentrations in the air, higher molecular weight does not necessary imply a lower detectability. Besides the mentioned possibility that some of them have to be sampled by contact, higher molecular weight is correlated, at least in humans, with increased odour intensity (Edwards and Jurs, 1989). If that happened in other species, animals may compensate for decreased concentrations by increasing their olfactory sensitivity to the chemicals being used.

The fading time of a scent can be modified by the carrier used. Faeces, for example, will release scents longer than urine, as they present a smaller surface/volume ratio, and thus evaporation is more difficult. In addition, the type of substratum on which a scent is deposited can affect the kinetics of odour release. Thus, a clay substratum releases volatiles more slowly than other substratums, (Alberts, 1992).

The size of the territory in which the scents are applied is likely to greatly influence the pattern of mark distribution. Territory marks should be deposited in sites and patterns such that their chance of being discovered is maximised (Gorman, 1990). Gorman (1990) found that hyenas (*Crocuta crocuta*) marked along

the boundaries of their territory when living in large groups that defended small, food-rich territories. However, the same species living in small groups that defended large territories containing poor food supplies, marked in many sites within their territory (hinterland marking). In this latter case, boundary marking would be excessively costly in energetic terms because the marking individual would have to travel frequently along the entire border. In hinterland marking, marks were concentrated around the parts of the territory with the highest food concentration and where the hyenas spent most of their time. It is thus, likely that these areas are better defended than other areas of their territory, as intruders have a greater probability of both detecting range marks and being detected by a resident.

Territory size should also affect the fading time scents will require to be effective in communication. Those species holding large territories are likely to deposit fewer marks per unit of area than species holding smaller territories. Since intruders have a lower probability of encountering a mark in a large territory than in a small one, species who have to mark larger territories should use longer lasting scent marks. However, there does not seem to be evidence for the existence of such adaptation.

The information content of the signal can also impose a constraint on scent composition. Signature systems for individual recognition need to be more complex as the number of interacting individuals increases because there is more room for mistakes (Beecher, 1989). Thus social animals should have more complex scent mixtures than solitary ones. This complexity does not seem to be reached by means of using larger, more complex molecules, as Alberts (1992) failed to find a significant difference between the

molecular weight of scents deposited by solitary and non solitary species.

In summary, the characteristics of the environment (in addition to those of the social system) are likely to have a strong influence on some of the features of both the scent marking deposition system and the composition of the scent.

1.3. Functions of scent marking in mammals.

Scent marking may play a number of roles in the ecology of mammals (reviewed by Stoddart, 1980, and Brown and Macdonald, 1985). I shall discuss possible functions for scent marking, such as incidental marking, orientation, advertisement of territoriality/ dominance, physiological and behavioural priming of conspecifics, recognition of gender, attraction of mates, assessment of their reproductive state, reproductive synchronization, discrimination of individuals, recognition of a group odour, alarm signals, and communication of diets. However, it should be noted that most functions are not mutually exclusive and scent marking (or any particular pattern of scent marks) could serve several functions at the same time. For example, marking by males in response to intruder males may also attract females.

1.3.1. Non communicatory role for the deposition of scents.

As discussed earlier, scent marking may have evolved from the ability of animals to gain information from products released by conspecifics for reasons other than for communication. However, some secretions from external glands and products released onto the ground may not have evolved for any communicatory function.

This may appear to be scent marking, although as defined earlier, it is not strictly so. For example, sebum glands, such as those found in birds, may serve mainly to lubricate skin or annexed structures, and urine may be deposited for no purpose at all other than voiding the bladder (incidental deposition). In the black tailed deer, *Odoicoleus hemionus columbianus*, the major compound released by the interdigital gland, (E)-3-tridecen-2-one, has a broad action against both fungi and bacteria (Wood, Shaffer and Kubo, 1995). Wood, Shaffer and Kubo (1995) suggested that this substance may serve as an antibiotic to control fur micro-organisms, or alternatively, that it may help to prevent bacterial breakdown of semiochemicals.

Mammals usually concentrate scent marks used for communication in specific sites. Rabbits and badgers, for example, use faeces in communication and concentrate them in latrines (Bell, 1980, 1981; Roper, Shepherdson and Davies, 1986; Roper et al., 1993). Thus, concentration of scents in specific sites may be interpreted as evidence that the scents are being used for communication. However, it is important to realise that such patterns could arise from incidental deposition of scents. For example, if individuals prefer particular pathways and deposit their urine incidentally, urine will have a higher probability of being deposited on those paths than in adjacent areas. Similarly, if an animal prefers to rest in the entrance of its burrow, or uses trees to scratch or to remove parasites, sebum could be concentrated in those sites. An animal may self anoint (Gosling, 1982) using a specific pattern of behaviour that would appear to serve for communication even if the sebum was used solely for lubricating or water-proofing the fur. In some cases these behaviours might lead to the assumption that the individual was range marking when it was

not actually doing so. Hence a non-uniform distribution pattern of scents, or a specific deposition behaviour does not necessarily imply that a scent is being used for orientation or social communication. On the other hand, the absence of an uneven pattern of marks or lack of specific scent deposition behaviour cannot be taken as a proof that scents are not being used for orientation or social communication. Scent marks may be deposited apparently at random, and nevertheless help the marking individual in orientation (to avoid leaving the familiar territory inadvertently), warning intruders, etc. The lack of a special behavioural pattern of deposition (e.g. rats often deposit urine as they move; personal observation), or failure to concentrate marks in certain places may thus lead again to a wrong conclusion, i.e. that the species does not make use of scents for any communicatory function.

Scents may be deposited in special sites or through particular behaviours to serve a hygienic, non communicatory function. Such may be the case of the aboriginal house mouse, *Mus spretus* (Hurst and Smith, 1995). Both males and females of this Iberian species of mouse pick up faeces with their mouth, often carrying them a few centimetres away before leaving them again on the floor. Individuals prefer to pick up their own faeces, and Hurst and Smith (1995) did not find any difference in manipulation whether or not the faeces were infected with parasitic tapeworms. They found that mice did not show any further interest in faeces once they had been pushed to one end of the test tunnel, and on several occasions, mice even pushed faeces through mesh caps at the ends of the tunnels. Furthermore, mice only seemed to be interested in fresh faeces. In this case, faecal manipulation seems to function just to push faeces out of the way, although it is still possible that faeces may play some

unexpected role in communication. Hurst et al. (1996) and Gray and Hurst (submitted) found that both *Mus spretus* and M. *domesticus* deposit faeces away from nest sites. This seems again a hygienic behaviour, although it may serve to avoid leaving cues that could be used by predators to track them.

1.3.2. Role of scent marking in Communication.

1.3.2.1. Orientation or self communication.

Scents could be deposited by a marking individual to help it to navigate around its home range. Some mammals, such as rodents, increase their marking behaviour for a limited period when they are first introduced into a new area, like a clean cage (Indian soft-fured rat, *Rattus meltada*, Idris and Prakash, 1987; in Norway rats: Anisko, Adler and Suer, 1979; Mink and Adams, 1980; Lee, Mitchell and Adams, 1984; Peden and Timberlake, 1990; in bank voles, *Cletrionomys glareolus*: Rozenfeld, Boulangé and Rasmont, 1987; in golden spiny mouse, *Acomys russatus*: Rozenfeld, Rasmont and Haim, 1994). This may serve to remind the individuals which area they have already visited and thus, increase familiarity with their new home range and/or to assist them in orientation. Male rats, for example, urinate within 30 seconds of their introduction into a clean test cage (McIntosh, Davis and Barfield, 1979). It could be argued that this is a fear response, but this does not exclude the former. Additionally, a fear response may elicit urine marking in order to increase familiarity with an area and consequently, help to reduce the fear response.

Often, scent marks are deposited in locations of special behavioural significance within their home range such as burrows, food caches, etc. In this case marks may be a means of chemically

labelling those areas. Wolves, for example, defecate more often along paths than elsewhere, and they direct raised leg urination towards vertical objects such as trees, kennel walls, grass tufts and fences (Asa, Mech and Seal, 1985). They use urine sprays to mark food caches which have already been depleted of their load (Harrington, 1981). Subsequently, marked caches receive little attention, but both unmarked caches containing food dug by wolves, or unmarked caches without food, dug by the experimenter, were actively investigated. In this case, scent marks help in orientation towards finding food. Similar results have been found in other carnivores (reviewed by Macdonald, 1980).

Scents do not need to be deposited in sites of special behavioural significance to assist in orientation. All individual mice living in a group (Hurst, 1989, 1990a, 1990b, 1990c) deposit urine on the substratum, creating a background against which any change can be detected. This may play a role both in orientation (Hurst 1989) and in social behaviour (Hurst 1990a, 1990b, 1990c, 1993).

Rats too appear to use scent marking in orientation by means of trails (see section 1.4.7.2.1).

1.3.2.2. Role of scent marking in social communication.

If scent marking for orientation can be regarded as self communication for use at a later period in time, communication with other conspecifics can be regarded as social communication.

1.3.2.2.1. Scent marking in competitive communication.

Vertebrates usually confine their movements within a particular area called the home range. Although individuals may not defend their home ranges in some species or in cases of great

abundance of scattered resources (Brown, 1970), competition usually forces them either to defend part of their home range against intruders (which is then termed a territory, see section 1.4.5.1) or to keep a minimum distance between themselves and other conspecifics or groups of conspecifics (Brown, 1970). Thus, they increase their chances of securing a sufficient level of available resources or reduce predation/parasite pressure.

Brown (1970) highlighted two sets of behaviour involved in the defence of a territory: i) behaviours to exclude or dominate conspecifics such as attack, chase, bite, etc.; ii) those involving advertisement of their dominant status, including scent marking.

1.3.2.2.1.1. Role of scent marking in signalling dominance.

Much of the attention to competitive marking has been directed to the role marking plays in the defence of a territory. Thus, the traditional explanation for scent marking has been that marks deter intruders (Uexküll and Kriszat, 1934). However, in practice, they rarely do so (Thiessen and Rice, 1976; Gosling, 1982; Roper, Shepherdson and Davies, 1985; Klingel, 1991). Gosling et al. (1996) have proposed a mechanism to explain this controversial hypothesis on territorial marking: he and his colleagues postulate that intrusion should be likely when benefits outweight costs. Because the intrinsic information encoded in scent marks is probably very generalised, they argue, an intruder will use the information from scents found in a territory if he is at a disadvantage relative to the owner (i.e., if the cost of intrusion outweights the benefits). In such case scents 'deter' intruders. If the benefits for the intruder match more closely or outweigh the costs,

the intruder should seek more specific information about the owner and risk encountering it. These, Gosling et al. (1996) argue, are the cases when scent marking does not deter intruders.

Alternative hypotheses have been proposed that scents intimidate intruders (Geist, 1965), or, likewise, the presence of the territory owner's marks increases its confidence which gives it an advantage in fighting (Gosling, 1982; in rabbits: Mykytowycz, 1973; Mykytowycz et al., 1976). Both intimidation and confidence hypotheses refer to motivation and therefore, to the proximate causes of scent marking (Tinbergen, 1963). Hence, they do not oppose the functional or adaptative analysis adopted in this thesis (Sherman, 1988). As a consequence, no discussion of motivation roles is attempted here.

However, as Ralls (1971) has pointed out, mammals scent mark not just to exclude others from their territory, but in most situations when they are dominant or intolerant toward other members of the same species. Territorial marking is thus included as a case of dominance advertisement. Animals may mark in a competitive context when they are dominant in a territory (territorial marking), dominant in a group, both of these, or to signal their preference of access to some resource (females, food, etc.). Dominant individuals, usually of high competitive ability, gain an advantage by scent marking because this will result in avoidance by most intruders and therefore, will result in reduced costs for the dominant individual to maintain their status (Hurst, 1993).

Gosling (1990) has proposed 3 mechanisms to signal social dominance using scent marks:

i) Intrinsic information within the mark could signal the dominance quality of the donor. Female laboratory rats, for example, appear to be able to discriminate between unfamiliar laboratory males differing in aggressiveness using the scents left by them (section 1.4.7.2.6). Dominant bank voles investigate and scent mark (with flank marking and anal dragging) urine and faeces from unfamiliar dominant individuals (i.e. with successful aggressive experience) more than the same scents from unfamiliar males without aggressive experience (Rozenfeld and Rasmont, 1991), suggesting that some chemicals may signal the fighting ability of the male.

ii) Individuals could learn to associate marks with a known dominant. Past experience often influences investigatory responses. Resident rats, for example, investigate urine marks from familiar intruders for longer than those from unfamiliar ones (Brown, 1992).

iii) The predominant marks in the substratum could be matched against the odour of the individual encountered to identify the dominant (scent matching hypothesis; Gosling, 1982).

Some of the predictions of the scent matching hypothesis are: i) the owner should remove or replace marks in the territory that are not his; ii) the owner should mark itself with the substances used to mark the territory to allow scent matching (if the source is not readily available for investigation on its body, as ano-genital secretions are); iii) the owner should make itself available for scent matching by conspecifics.

Gosling's scent matching hypothesis is supported by several lines of evidence:

i) *Scent counter-marking of competitor's scents by residents occurs.* Although, in some species, scent counter-marking seems to function

to establish and strengthen pair bonds, as in wolves (Mert-Millhollen, Goodmann and Klinghammer, 1986; Rothman and Mech, 1979), in others, counter-marking seems to mask the marks of possible competitors. In badgers, *Meles meles*, individuals mark more at latrines where neighbours mark (Roper, Shepherdson and Davies, 1985), and faecal deposition is more common when recent faeces have been deposited (Roper et al., 1993). Resident Norway rats deposit urine marks on top of intruder urine marks in a competitive situation (Adams, 1976), while dominant rabbits, *Oryctolagus cuniculus*, defecate over conspecific urine samples (Bell, 1980). Scent counter-marking has been best studied in the golden hamsters, *Mesocricetus auratus*. In a recent study, Johnston, Chiang and Tung (1994) showed that vaginal and flank over-marking masks the scent marks of the previous individual. Masking is effective even if the top scent does not entirely cover the bottom scent (Johnston, Munver and Tung, 1995). However, it should be noted that the primary scents used in these experiments were laid down by the authors, which may elicit different responses to those produced by marks laid down by hamsters. Scent counter-marking will be discussed in more detail in section 1.3.2.3.

ii) One prediction resulting from Gosling's hypothesis but which Gosling himself did not explicitly make, is that anosmic individuals will be prevented from matching scents and anosmic territory owners should be less aggressive towards intruders than intact owners if they use their own odours to detect that they are "at home", since individuals are likely to be less aggressive when they are in an unfamiliar home range than when they are defending their territory. However, any test of this prediction and the interpretation of the results should be conducted with great caution,

as, on the one hand, impairing any ability of an individual may have multiple and unexpected effects in their social behaviour, and on the other, a territory owner is likely to be familiar with its territory and thus, it may not rely heavily on scents to detect its own home range.

In agreement with this prediction, *anosmia precludes the onset of aggression* both in wild (Alberts and Galef, 1973) and laboratory resident rats (Flannelly and Blanchard, 1982), or reduces resident's aggression towards intruders compared to aggression shown by intact residents (Flannelly and Thor, 1976a). However, this effect does not appear to arise from a general impairment in social behaviour, as anosmia does not appear to impair mounting behaviour (Thor and Flannelly, 1977).

iii) The best evidence on the existence of scent matching so far appears to be a test conducted by Gosling and McKay (1990). They placed unfamiliar, previously isolated males on either side of cages divided in two by a mesh barrier, to assess their aggressive behaviour. The background of scent marks was manipulated in such a way that one of the individuals, 'the resident', was surrounded by his own scent marks, whereas the other, 'the intruder' was surrounded either by the resident's marks or by marks from an unfamiliar male not present in the arena. The latency to attack by the intruder was greater and the number of fighting attempts was smaller when the substratum odour matched that of his opponent.

Hurst (1993) has proposed an alternative mechanism by which scent marks would help a dominant individual to keep its social status. Individuals may use all the information available concerning the individual identity, social status and their prior experience with

the mark donor. Thus, individuals would scent match the predominant marks on the substratum with the odour of the individuals encountered to identify the dominant. However, Hurst (1993) found evidence suggesting other mechanisms of olfactory communication working simultaneously with scent matching. Hurst (1993) suggests that the challenges for dominant status by some intruders despite their ability to identify the dominant meant that intruders used information from marks in the resident's territory to assess his ability to successfully defend his territory, and therefore, the intruder's chances of taking over. For example, intruders may use the presence of fresh intruder marks in the territory as an indication that the male is not defending his territory successfully.

1.3.2.2.1.2. Territoriality/dominance advertisement aimed at individuals of other species.

Animals may compete with individuals from other species and scent mark in response to their cues. Coyotes increase marking in areas where they live together with wolves, although the reverse is not true (Paquet, 1991). Bank voles, which are strongly territorial, counter-mark with urine and faeces in response to urine and faeces from another species of vole, *M. arvalis*, or from the wood mouse, *Apodemus sylvaticus*, (Rozenfeld and Rasmont, 1991), both of which occur in the same areas as the bank voles.

1.3.2.2.1.3. Scent marking by non-dominant individuals.

The previous hypotheses on competitive scent marking do not explain the role that scent marking by members of a group other than the dominant male may play. In some species dominant individuals do not exclude others, but live in a group occupying the

top position in a social hierarchy. Mice, for example, live in hierarchically organised groups, although individuals may establish exclusive territories, depending on resources, space available, etc. (Poole and Morgan, 1976). Rats live in a similar social system (see section 1.4.5.1.). In such group-living species, it is common for females also to deposit scent marks (Thiessen and Rice, 1976; in mice: Hurst, 1990b; in *Rattus meltada*: Idris and Prakash, 1987; in bank voles: Rozenfeld and Rasmont, 1991; in *Rattus norvegicus*: Calhoun, 1962), as do subordinates (in mice: Hurst, 1990a; Hurst, 1993; Hurst, Fang and Barnard, 1993; in hippopotami: Klingel, 1991; in badgers: Roper et al., 1993; in rabbits: Sneddon, 1991).

In cases where the individuals live in groups in which one individual dominates the rest, the dominant individual marks at a rate higher than the others (Ralls, 1971). Some examples are wolves (Asa, Mech and Seal, 1985; Mert-Millhollen, Goodmann, and Klinghammer, 1986; Asa, et al. 1990), rabbits (Bell, 1980), rhinoceros, *Ceratotherium simum,* (Owen-Smith, 1971), hippopo-tami, *Hippopotamus amphibius,* (Klingel, 1991), and mice (Hurst, 1990a, 1990c, 1993). Sometimes the dominant male is not the only one involved in the defence of the territory; females may also contribute, as is the case in the roof rat, *Rattus rattus*, (Ewer, 1971). In cases like this, scent marking by other defending members of the group may also serve as a substitute for aggression.

However, scent marks may also play other roles, such as maintaining tolerance between individuals of the group. Such is the case in mice, where both dominant and subordinate resident males

become more aggressive towards a familiar subordinate that ceases to contribute fresh odours to the home substratum (Hurst, Fang and Barnard, 1993).

1.3.2.2.1.4. Roles of scents in physiology priming.

Scent marking by members of the group other than the dominant male may play a non-territorial but still a competitive role. Competition does not need to be directed only to intruders, it can also arise within the group itself. Thus, breeding female mice, for example, mark at a higher rate than non breeding females (Hurst, 1990b), apparently to advertise their breeding status. In badgers too, females may mark to compete for breeding vacancies within the group, as well as to defend pups against intruders (Roper, et al., 1993). In bank voles, females increase marking and aggression towards other females near parturition (Rozenfeld and Denoël, 1994).

In most of these cases, scents probably act as primer pheromones, inducing long term physiological changes usually related to reproduction (reviewed by Brown, 1985a). Generally the individual inducing such physiological changes in its competitors gains a reproductive advantage from it. Thus, vaginal opening and first oestrus is delayed in female mice from 21 days of age when they are exposed to bedding impregnated by juvenile or adult females housed in groups of 4-6 individuals (Drickamer, 1974). Breeding inhibition mediated by scents is not only limited to juveniles. Champlin (1971) found that

isolated adult house mouse females, *Mus domesticus*, exposed to bedding soiled by a group of 4 adult females had significantly fewer oestrus cycles.

As pointed out in previous sections, the reader of the signal (whether chemical or of some other kind) responds to the signal for its own benefit. In consequence, primer effects of scents should not be viewed as an enforced inhibition of the weaker individuals by the most dominant, but rather as a way that individuals optimise their reproductive success in life history (Vandenbergh and Coppola, 1986; Drickamer, 1989). Vandenbergh and Coppola (1986) suggested that puberty pheromones appear to act as cues to the social environment that determine the maturational rate most appropriate for the conditions under which they are released. Drickamer (1989) found that female mice appear to seek odour cues, presumably to prime themselves.

1.3.2.2.1.5. Behavioural indicators suggesting that scents play a role in competitive advertisement.

Ralls (1971) has pointed out a number of lines of evidence that suggest when a scent is involved in communication among competitors:

i) The dominant individual usually marks at a greater rate than other individuals of the group. There are many examples of this, some of which have already been pointed out (section 1.3.2.2.1.3). Frequently, the scent glands involved are more highly developed in such individuals. In rabbits where males faecal mark at latrines, they have larger anal glands than females (Mykytowycz and Gambale, 1969;

Mykytowycz, 1970), although these studies do not include any comparison among males of different social status. In mice, on the other hand, dominant males produce urine at a greater rate than subordinates (Drickamer, 1995) and urine mark at a considerably greater rate (Desjardins et al., 1973).

ii) Males usually mark more than females. This appears to be very widespread among mammals (reviewed by Thiessen and Rice, 1976). However, this does not necessarily imply that scents are used in communication between competitors. Males may be marking to attract mates, or advertise their qualities. As they are generally the sex investing less in parental care, and females are choosier (Partridge and Halliday, 1984), males are more likely to advertise for mates.

iii) Individuals increase marking after encounters with neighbour or unfamiliar individuals. This finding is not conclusive, as such encounters may be sexually motivated. However, dominant individuals may regard some individuals of the opposite sex as competitors, as is the case in mice (section 1.3.2.2.2.3).

iv) Many species mark in response to individuals of their own sex more than in response to individuals of the opposite sex. Although some of the previous behaviours may be due to both competitive and sexual advertisement, a greater marking response towards individuals of the marking individual's own sex cannot be explained as sexual marking. Such is the case in the bank vole, where dominant males investigated, flank marked and dragged genitals (also regarded as scent marking) more in response to urine and faeces from

unfamiliar males than to those from females (Rozenfeld and Rasmont, 1991).

v) Scent marking appears in association with situations where aggression occurs. This could happen in a number of situations:

i) *Marking usually precedes or follows aggression* (Thiessen and Rice, 1976; in coyotes: Wells and Bekoff, 1981). Dominant hippopotamus bulls holding neighbouring territories display ritualised defecation simultaneously at their common boundary (Klingel, 1991). Intruder laboratory rats mark less after being defeated (Adams, 1976), while wild male *Rattus rattus* intruders scent mark after winning a contest (Ewer, 1971), as happens also in mice (Desjardins et al., 1973). In mice, increasing the marking rate of a subordinate individual experimentally elicits dominant male attack specifically towards the marks' donor (Hurst 1993).

ii) *Territory owners reduce or cease scent marking after a defeat.* For example, rhinoceroses cease spray-urinating and gradually stop dung-kicking after being defeated (Owen-Smith, 1971). Similarly, urine marking in bank voles ceases almost completely 14 days after being defeated by another male (Rozenfeld, Le Boulange and Rasmont, 1987).

In bank voles, urine marking is correlated with aggression among females and females increased both marking and aggression when a male was introduced (Rozenfeld and Denoël, 1994). Urine marking in this situation may be a signal aimed at the male and hence a sexual signal, although its occurrence simultaneously with aggression suggests that it may also be a competitive signal.

1.3.2.2.2. Scent marking in sexual communication.

Scent marking may play a number of roles in sexual communication. In the following paragraphs I will discuss the evidence suggesting that scents are involved in sexual communication. Then I shall discuss the roles that scent marking may play in sexual communication and the evidence. Finally I will discuss the possibility that scents may play simultaneously roles in sexual and communication between competitors.

1.3.2.2.2.1. Evidence suggesting the involvement of a scent in sexual communication.

i) One of the sexes marks more than the other. In a review on mammalian scent marking Thiessen and Rice (1976) indicated that marking is usually sexually dimorphic. Although greater marking by one sex may be involved in sexual communication, any sex bias in scent marking does not necessarily imply this type of communication. For example, greater marking by one sex could derive from its use in advertising the defence of the territory rather than in attracting mates. Thiessen and Rice (1976) indicated that females usually mark more when receptive. Such increase in female scent marking at oestrus or pro-oestrus strongly suggests that scent marking is involved in sexual communication, although in some cases the signal may be aimed at other female intruders which may compete for breeding vacancies, such as in bank voles (section 1.3.2.2.1.5).

ii) Greater response towards scents from the opposite sex. Another line of evidence strongly supporting a role for scent marking in sexual communication is that investigation and counter-marking towards scents from the opposite sex is stronger than

towards those from the marking individual's own sex. Meadow voles, *Microtus pennsylvanicus*, investigate the scent from conspecifics of their opposite sex more than those of their same sex, around the mouth, anogenital and posterolateral region, and their faeces and urine (Ferkin and Johnston, 1995a). Much of the research on scent preferences in laboratory rats conducted under laboratory conditions shows a stronger response towards urine from individuals of the opposite sex (section 1.4.7.2.6), which is not surprising considering that these rats are usually housed in single sex groups with little or no access to females.

iii) Scent marking increases during sexual encounters. For example, scent marking increases in some species during copulation. Male coyotes mark during courtship (Wells and Bekoff, 1981). The male laboratory rat returns to the same place after every ejaculation and urinates (Anisko, Adler and Suer, 1979; McIntosh, Davis and Barfield, 1979). Although this urination could feasibly serve a hygienic purpose, by removing possible genital parasites transmitted from their partner, other evidence suggests that this urination is a form of scent marking (McIntosh, Davis & Barfield, 1979): i) male rats also urine mark after a female is first introduced into the experimental cage; ii) both male and female lick the male urine and; iii) during copulation, the female returns to the male urine puddle to sniff before returning to the male.

iv) Scent marking is under the control of sexual hormones. This does not represent conclusive evidence because both aggression and sexual behaviour are under control of sexual hormones.

It has already been pointed out that the changes in oestradiol and progesterone leading to behavioural receptivity result in

increased marking by females (Thiessen and Rice, 1976). Gonadectomy, which reduces sexual behaviour, also affects both scent marking and olfactory preferences. Thus, ovariectomised female laboratory rats urine mark less than intact females and do not show preferences when investigating urine from intact or castrated males (Brown, 1977; Matochik, Barfield and Nyby, 1992). Similarly, castrated male rats urine mark less than intact males (Price, 1975, 1977; Brown, 1977; Taylor, Bartko and Farr, 1987; Matochik and Barfield, 1991) and do not prefer to investigate male vs female nor castrated vs intact conspecific urine samples (Stern, 1970).

v) Factors affecting olfaction also affect sexual behaviour. Additional evidence strongly supporting the involvement of scents in sexual communication regards the effect of anosmia on sexual motivation. Anosmia reduces or precludes male interest in females (in wolves: Asa et al., 1986). Similarly, sexual experience may affect olfactory preferences, with individuals without sexual experience failing to show olfactory preferences (Carr, Loeb and Dissinger, 1965; Stern, 1970; Lydell and Doty, 1972; Brown, 1991).

1.3.2.2.2.2. Scent marking roles in sexual communication

Scents may play a number of roles in sexual communication.

Gender recognition. The simplest role a scent may play in sexual communication is to provide cues about the gender of the mark's donor. The evidence showing that animals can recognise the gender of an individual from its scents is overwhelming: all tests indicating an investigatory or marking preference for one sex over the other imply sex discrimination (reviewed by Ralls, 1971; sections 1.3.2.2.1.5, and 1.3.2.2.2.2, in rats: 1.4.7.2.5 and 1.4.7.2.6). Ferkin

and Johnston (1995a) have indicated that some scent glands may be specialised in the assessment of sex identity and reproductive status. They found that meadow voles investigated scents from conspecifics of their opposite sex more than those of their same sex when these scents were faeces, urine, anogenital scents, scents from the posterolateral region or mouth. However, they did not discriminate when the scents used came from the back, chest, head-neck-ear region or feet. Rats can discriminate gender in the urine even when it comes from new-born pups (Moore, 1985).

Mate attraction. One of the traditional explanations for scent marking is that it serves to attract mates. Vaginal secretions in the female golden hamster advertise receptivity and attract males (Johnston, 1985). It is also very common to observe in any city the attraction that female dogs and their urine produce on male dogs. The scent is such a powerful stimulus that it may encourage a male dog to enter at the first opportunity into a usually closed building, climb up several storeys and wait on the doorstep of a flat housing a bitch (pers. observ.).

Assessment of reproductive state. Males use scents to assess the reproductive state of females. The best known example of assessment of female reproductive state by males has already been discussed: detection of oestrus. However, males appear to detect more female states than just oestrus or non-oestrus. Ferkin and Johnston (1995b) found that meadow voles showed no preference for anogenital scents of a reference female (non-oestrous non-pregnant) compared to those of a pregnant or lactating female except during two periods: i) males avoided scents from the pregnant female on days 19-20 of gestation, just prior to parturition (some rodents, including rats, are highly aggressive during late pregnancy, see

section 1.4.5.1): ii) during postpartum oestrus (days 1-2), when the female was receptive, males were attracted to the odour of a lactating female.

Reproductive synchronization and stimulation of mates. Scents may prime the physiology of possible mates in order to synchronize the reproductive state of both individuals in the pair. It has been cited earlier that male coyotes mark during courtship (Wells and Bekoff, 1981), as is the case in wolves (Rothman and Mech, 1979). Rothman and Mech (1979) suggested that such increases in marking may help to synchronise both mates, although a number of alternative hypotheses would predict the same result (such as territory formation, securing resources, etc.). Bedding scented by an adult male laboratory mouse may prime the physiology of females, inducing oestrus in anoestrus females (Whitten, 1956). A similar effect has been found in female laboratory rats (Antz-Vaxman and Aron, 1986; see section 1.4.7.2.6). Similar effects can be found in normally cycling female rats: male scents may shorten the female oestrous cycle and female scents produce oestrous synchrony in grouped females (section 1.4.7.2.6). Scents may also accelerate puberty in juvenile females, the so called Vandenbergh effect. Female mice housed from 21 days of age or from birth to 21 days of age in a cage with bedding scented by adult males have their first oestrus earlier than females without such stimuli (Vandenbergh, 1969; Fullerton and Cowley, 1971). Individuals appear to be sensitive to the social rank of the scent donor. Application of urine from dominant male mice to the nares of 28 day old females for seven days produces a significant increase in uterine weights compared with either a water control or urine from subordinates (Labov, 1981).

Mate assessment and mate choice. If one sex invests more in offspring than the other it will become a limiting factor, and thus, the individuals of the sex investing least are likely to compete amongst themselves for an opportunity to mate (Partridge and Halliday, 1984). This will allow the sex investing more, usually the females, to be choosier than their counterparts. Scents may be used for such mate assessment.

Marr and Gardner (1965) found indirect evidence of the involvement of scents in mate choice, although in this case it was studied in males. Pup rats reared with perfumed mothers prefer similarly perfumed rats as mates when adults. If adult male rats reared with perfumed mothers are allowed to mate only with rats bearing a natural odour their mating performance is reduced compared to males reared with naturally scented mothers. Unfortunately both types of males were not tested with perfumed females to assess whether the converse was true. This impairment of mating performance when mates smell very different from mothers may reflect Bateson's (1982) hypothesis that animals prefer individuals only slightly different to them to obtain an optimal balance between inbreeding and outbreeding.

Scents might be used for mate assessment at a variety of levels: assessment of socinl status, competitive ability, genetic quality (e.g., resistance to parasites), assessment of physiological quality (body size, health state, etc.), which may depend on the quality of the resources, etc. These will be discussed in following paragraphs.

It is likely that females will choose males according to their resource holding potential, which is usually correlated with social status. The common finding that factors affecting mate choice affect olfactory preferences suggests that scents are involved in selecting

mates. Female rats visit more frequently, spend more time with, and mate more frequently with an unfamiliar dominant male of a dyad than with an unfamiliar subordinate (Carr et al., 1982). When mating in groups, female rats mate for longer and facilitate sperm transfer of the dominant male over the subordinate one (McClintock, Anisko and Adler, 1982). These preferences are also evident in the time spent investigating male scents. Female rabbits, for example, investigate urine from high ranking unfamiliar males for longer than that from low ranking unfamiliar ones (Bell, 1981).

Choosing a high quality mate is likely to enhance female reproductive success (Partridge and Halliday, 1984). Thus, if olfactory preferences reflect mate preferences, mating with the donor of the preferred scent should result in increased reproductive performance. That is the case in female golden hamsters. Females allowed to mate with a male whose scents (soiled bedding) they had investigated previously have larger litters than other conspecific females who were mated with a male different from the donor of the odour that they had been allowed to investigate (Tang-Martinez, Mueller and Taylor, 1993).

Scent marks may indicate the parasite load of an individual, and thus, might indirectly show the genetic quality of a possible mate expressed as their resistance to parasites. Female mice prefer an area marked by a healthy male compared to one urine marked by a male carrying the nematode parasite *Heligmosomoides polygyrus* (Kavaliers and Colwell, 1995). A curious finding of Kavaliers and Colwell (1995) was that females stayed for a similar period of time in a clean area (which elicits strong neophobic responses) and that with urine from a parasitised male, which suggest avoidance of the parasitised urine.

1.3.2.2.2.3. Inter-sexual communication is not always sexual communication.

Most of the evidence shown so far may lead to the oversimplified conclusion that all communication between males and females is sexually motivated, and that all intrasexual communication has a competitive function. However, both behaviours are linked and scents may play a communication role in both at the same time. The predominance of evidence showing that communication between sexes is commonly a sexually motivated one has probably arisen because authors were looking for a role for communication between the sexes in reproduction. Hence, they used isolated individuals, generally from laboratory strains, and brought some of the females into oestrus with the aid of oestradiol and progesterone benzoate. A male with no competition for food or territory, little or no sexual experience and facing a soliciting female in heat in a strange cage that he does not regard as his home range or territory, is very likely to be sexually motivated. However, under more naturalistic conditions, where males may be living with established mates in their usual home range, it is more likely that intruder females are regarded as competitors. Territorial male mice, for example, regard outsider females as intruders and they actively exclude them from their territories (Reimer and Petras, 1967; Hurst, 1990c). During a study of free-ranging black rats, *Rattus rattus*, most intruders were actually driven off by females, including males larger than the attacking female (Ewer, 1971). Although there is relatively little aggression between the sexes in Norway rats, both kinds of intersexual aggression have been reported. In captive colonies of wild rats, female intruders were observed to receive attacks by

dominant males (Barnett, 1958; Barnett, Dickson and Hocking, 1979). Female wild rats lactating in large open enclosures (Calhoun, 1962), and laboratory rats kept in smaller enclosures (Brain et al., 1980; lactating laboratory females: Flannelly, Flannelly and Lore, 1986; Flannelly and Flannelly, 1987) show aggression towards both resident and unfamiliar males. Cohabitation with males also triggers aggression towards intruder males in females prior to parturition (Albert et al., 1988).

A finding that suggests an inextricable link between competitive and reproductive communicatory roles is that, as mentioned before, both aggressive and sexual behaviours are under the control of sex hormones. Sex hormones also control scent marking. Thus, as mentioned earlier, female rats increase scent marking when they are receptive (Calhoun, 1962; Birke, 1978; Birke and Sadler, 1984; Lee, Mitchell and Adams, 1984; Matochik, White and Barfield, 1992; but see Peden and Timberlake, 1990), and they reduce their marking rate and show no preference for intact over castrated males after ovariectomy (Brown, 1977; Matochik, Barfield and Nyby, 1992). On the other hand, castrated female rats do not show aggression towards males (Albert et al., 1989a) in contrast to intact females. Males decrease urine marking (Price, 1975, 1977; Brown, 1977; Taylor, Bartko and Farr, 1987; Matochik and Barfield, 1991) and show no investigatory preference for females after castration (Stern, 1970). Castrated males also receive fewer attacks than intact males (section 1.4.5.1). The onset of puberty leads to an increase in sex hormones and, in laboratory rats, triggers in the maturing male the establishment of preferences for scents from the opposite sex (Carr, Wylie and Loeb, 1970) and elicits the aggression of mature males towards him (Thor and Flannelly, 1976a).

This ambivalence of scent marking in communication has also been observed in the wild. Many animals increase marking during the breeding season. In mice, breeding females mark at a much higher frequency than other females (Hurst, 1990b). Wolves mark and counter-mark more when breeding (Mert-Millhollen, Goodmann and Klinghammer, 1986). Badgers too increase faecal marking during the breeding season (Roper et al, 1993). Adult rabbits increase their frequency of visits to latrines, and presumably their frequency of marking, in April, their breeding season (Sneddon, 1991). Female coyotes mark more during the denning season (Wells and Bekoff, 1981). In water voles, *Arvicola terrestris*, no latrines are found at peripheral population sites, but they are present in core sites, where breeding occurs, and only during the breeding season (Woodroffe, Lawton and Davidson, 1990). During this period, competition is likely to concern access to mates and the resources required for breeding. Thus, if latrines play a role in communication, they may be produced by males to attract mates, or by females to advertise their receptivity (i.e., a role in sexual communication). But latrines may also constitute an aggressive display by males to secure a territory against intruders, or to defend mating access to resident females. As a scent may have different meanings for different individuals (see section 1.1.1), scents deposited during the breeding season may have a meaning concerning the threat of aggression towards individuals of the same sex, but may, at the same time, attract mates. Female bank voles may form latrines as a means of territorial advertisement. They are strongly territorial during the breeding season (Rozenfeld and Rasmont, 1991), the time when they increase scent marking in latrines.

1.3.2.2.3. The role of scents in individual recognition.

Scents also play a role in individual recognition. There is abundant evidence that many different species of mammals can discriminate between two individuals using their scents (reviewed by Halpin, 1986). For example, female rats, habituated to the odour of one male, prefer the odour of a new male (Krames, 1970). However, as Halpin (1986) has pointed out, such type of discrimination does not imply discrimination between individuals, but between individual scents. There are two lines of evidence showing that some species may recognize individuals and not just their scents:

1) Golden hamsters. Males habituated to vaginal smears of a female, investigated the flank scent from the donor less than that from a different female (Johnston and Jernigan, 1994). This test shows that male golden hamsters discriminate between individuals, and not just individual scents. In this case, the scents used after the habituation period were completely different to that producing habituation. Thus, the habituation shown from a different scent from the same individual indicates that golden hamsters recognise the individual from one of its scents.

Scents from different sources may be specialised for individual recognition. After habituation to eight types of scents from the same male and one from the same female, male golden hamsters showed increased investigation when presented with the same type of scent from a different individual for five of the eight scent types. These were: male flank gland, urine, faeces and ear gland, and female vaginal secretions (Johnston et al., 1993). However, Johnston et al. (1993) found that another four scents (those from saliva, feet,

behind the ears, and flank from flank glandectomised males) do not appear to be used for individual discrimination in hamsters.

2) Mice. However, as pointed out by Halpin (1986), these kind of tests do not show whether individual recognition works in a natural situation. Such a test has been conducted by Hurst (1993) in a captive colony of wild male and female mice. Increasing experimentally the proportion of marks from a subordinate male triggers aggression of the dominant specifically towards the donor of the marks. This test not only shows that individual recognition takes place in a semi-natural situation, but also that it is important for the establishment of dominance. Hurst (1993) also found that marks from unfamiliar subordinates increases general aggression between subordinates of the colony. Hurst suggested that this may be due to a difficulty in distinguishing between odours from mice of similar status.

Halpin (1986) has suggested a number of roles that individual recognition may play in communication. One has already been pointed out: recognising the dominant individual, or those with which the investigating animal had a negative experience, may help in reducing the cost of fight injuries. Additionally, individual recognition is of primary importance for the dominant individual if scent marking is to produce avoidance by conspecifics and thus reduce challenges to him/her. Halpin (1986) suggested that individual scent recognition may play a role in helping an animal to become familiar with an area; to recognise mates (and thus reduce attacks towards them); in mate selection (where the selected individual must be recognised from its scents); and discrimination of own group members and/or kin (discussed more thoroughly in section 1.3.2.2.4).

1.3.2.2.4. Other roles for scent marks in olfactory communication.

In the following paragraphs I shall discuss additional roles of scent marking that have been studied less often such as the creation of a group odour, alarm signals, the possibility that scents are used to infer the density of individuals in an area, and the communication of diets.

Group odour. Scents may play a role in creating a group odour allowing individuals to be recognised as members of a particular group. However, the evidence seems to be very scarce in most species. Rabbits produce latrines which are visited mainly by dominant males, but also by other members. Females sit in latrines but apparently do not mark (Sneddon, 1991); and Sneddon (1991) has suggested that this might serve to allow females to acquire a group odour. Rats mark conspecifics with urine during a behavioural sequence termed 'crawling over' (Taylor et al., 1984; Taylor, Bartko and Farr, 1987; Taylor, Griffin and Rupich, 1988). This might function to create a mixture of group odour in the fur of all members of the deme. Additional evidence is found in mice. It has already been mentioned that all members of a group of mice living within the same territory mark the substratum of their home range (Hurst, 1989, 1990a-c). This might serve as a group odour for individuals to be recognised as residents. Dominant male mice exclude intruders from their territory (Hurst, 1990c). Both dominant and subordinate residents are more aggressive towards individuals who cease to contribute to this background mixture than towards an equivalent member which contributes to this group odour (section 1.3.2.2.1.3). Furthermore, Hurst and Barnard

(1992,1995) found that group membership discrimination by means of olfactory cues is a key factor in determining social tolerance in mice. One of the few studies testing biochemically differences in scents is that of Davies, Lachno and Roper (1988) in the anal gland secretion of badgers. They found a difference between chromatograms of anal scents from captive and wild populations of badgers (ie. housing regime was reflected on the composition of this scents), and a suggestive (but statistically untestable) difference between dyads of females from different wild groups.

Kin discrimination. Scents seem to play a role in kin discrimination. Aldhous (1989) found that male juvenile laboratory mice could discriminate siblings from cross-fostered littermates using olfactory cues. Aldhous found also evidence supporting a group odour hypothesis, because exchange of odour cues among juveniles led to a misidentification of cross-fostered juveniles as kin by adults. Furthermore, Hurst and Barnard (1992, 1995) found that olfactory discrimination of group membership was more important than kin discrimination with respect to social tolerance. Hurst, Fang and Barnard (1994) found that relatedness reduces scent differences between individuals and thus it reduces discrimination. Because mice tend to inbreed within groups, discrimination of changes in the olfactory background is then more difficult and this, in turn, increases the probability that a related non-member individual will be accepted. Hurst, Fang and Barnard (1994) point out that this reduced discrimination appears as kin-biased tolerance when it is actually not. They argue that findings on kin discrimination in mice are an artefact of group member discrimination (Hurst and Barnard, 1995), and that this might also be the case for other mammal species.

Stress and warning. One of the first functions studied in olfactory communication in insects and non-mammalian vertebrates was the use of scents as alarms. Evidence seems to be especially scarce in the case of mammals. One such case is the black-tailed deer, *Odoicoleus hemionus columbianus*. Its metatarsal gland produces a garlic-like odoriferous substance when the deer are alerted, chased, cornered or brought into strange surroundings (Müller-Shwarze, 1987).

Assessment of population density. Scent marking could also assist individuals to assess the density of the local population. As discussed earlier, this could, in turn, help them to modulate their reproductive physiology in accordance with each individual's opportunity to breed (Vandenbergh and Coppola, 1986; Drickamer, 1989). Davis proposed in 1949 that rats secured enough resources through aggressive behaviour, keeping the number of individuals in their home range under carrying capacity, and that communication would be essential in such a process (Davis, 1987). Telle (1966) suggested that the possibilities for a rat of being accepted in a group depended on the group's size. A migrating individual might use scents on the substratum to assess the size of a rat group, and thus assess its possibilities of being accepted.

Communication of diets. Finally scent marking and other types of olfactory communication may play a role in the social learning of conspecific diets. The best known example is Galef's work on rats, which will be discussed in section 1.4.6.

1.3.2.3. Scent counter-marking.

Many mammals deposit scents after investigating those previously deposited by conspecifics. This is termed scent counter-marking.

The functions of scent counter-marking seem unclear. According to Gosling's hypothesis on territorial marking, the territory owner should remove alien marks (Gosling, 1982). Counter-marking could be a form of covering up intruder's marks. Several examples have been cited in which scent counter-marking appears in an aggressive context, such as among wolves (Peters and Mech, 1975), rats (Adams, 1976), and mice (Hurst, 1990a, 1990b, 1990c, 1993). Bank voles counter-mark not just scents from their conspecifics, but also those from other vole species with urine and faeces (Rozenfeld and Rasmont, 1991).

In some instances, counter-marking seems to play a non-territorial role. The dominant male and female pair in a pack of wolves will counter-mark each other's urine. It has been suggested that, in this case, urine counter-marking may serve to keep and advertise the pair bond (Mert-Millhollen, Goodmann and Klinghammer, 1986). Female wild rats flank-mark objects and burrow entrances the night before they come into oestrus (section 1.4.7.2.6). Males counter-mark these scents (Calhoun, 1962). In this case counter-marking appears to play a role in sexual communication (such as indicating readiness to mate with the female), although it could also serve to mask the female's scent so that competitors would not be attracted to it, and, hence, reduce competition from other males. These counter-marks may also serve to signal that the counter-marking male has exclusive or priority of

access to the female, although this explanation seems unlikely because swarms of males end up following the oestrous female.

After new marks are deposited over old ones, Johnston, Chiang and Tung (1994) proposed that three things might happen:

1.-Scent blending may occur. In this process both scents would blend to create a new, different scent. Thus, none of the initial scents would be recognisable in the blend.

2.-Individual scents may remain distinct and identifiable.

3.-Scent masking may occur. The new scent may mask the previous scent and its information would be lost.

Johnston, Chiang and Tung (1994) found that male golden hamsters habituate to the top scent, but not to the bottom scent in a counter-marking sequence laid by the authors when tested using vaginal smears from different individual females. The top scent thus appears to mask the bottom scent. If counter-marking in a competitive situation physically masks signals, as overlapping between the songs of neighbour birds does, the individual depositing the counter-marks could be showing that it will no longer use the information in the counter-marked scents nor will allow other potential receivers to use that information (McGregor et al., 1992). Hence, counter-marking may work as a way of escalating a contest.

However, in other scent-marking systems, such physical masking seems implausible. Badgers, for example, seem to counter-mark using faeces (Roper et al., 1993). It is difficult to imagine how one faecal deposit could mask another one. In cases where physical masking seems unlikely, a semantic masking may occur. Thus, counter-marking may cancel the meaning of previous marks deposited under the current one, without the need of physical masking. Suppose the message conveyed by marks indicates that

the individual whose marks are deposited on the top is dominant, then the dominant individual would need only to counter-mark the intruder's marks to deprive them of meaning. However, the only report apparently published on partial overlapping in counter-marks is yet more striking. Even though golden hamsters were presented during a habituation phase with two vaginal smears which were only partially overlapping, so that individuals could sniff at each mark separately, they habituated only to the top scent (Johnston, Munver and Tung, 1995). When both scents were presented separately, golden hamsters investigated the top scent as much as the partially overlapping marks, suggesting that they regarded them as familiar. The bottom scent was, however, investigated more than the mixture of marks and as much as an odour from a novel individual. This suggests that the bottom scent was regarded as unfamiliar, *even though it was not completely masked!* Thus, golden hamsters seem to have a selective memory only for those scents whose meaning is not yet outdated. This could be an adaptation to obtain and keep only important information from scent-mixtures which might be of various ages and built up by a number of conspecifics.

Masking seems a suitable process in communicatory competition because the scent at the top hides the information below it. However, the other two physical processes that may happen in counter-marking, i. e., the creation of a new blend or the mixing of odours which keep their individual distinction, do not hide information from other scents and seem less suitable for roles in competitive signalling. Thus, they seem more likely to play a role in creating a group odour, a pair bond and other non-competitive functions.

1.3.2.4. The role of the vomeronasal organ in chemical communication.

The vomeronasal organ is a small narrow and elongated structure sited in the floor of the nasal region (Romer and Parsons, 1986). The vomeronasal system (the vomeronasal organ and the accessory olfactory bulbs) communicates with areas of the brain involved in reproduction (Wysocki, 1979). Severing the organ in males impairs their ultrasonic calling in response to female conspecifics or their odours, and reduces the rate of marking to that shown in response to males (Wysocki, 1982). Food finding is not impaired, however. As discussed later (section 1.4.7.1), ultrasonic vocalisations are involved in both mating and aggression. Thus, male impairment of calling in response to scents from females implies that the vomeronasal system may be involved in detecting sex scents. In mice, males without vomeronasal organs urine mark at half the rate of intact males, and initiate attacks on only 50% of occasions compared to intact males (Maruniak, Wysocki and Taylor, 1986). In laboratory rats, the increase in ovulation rate in response to male scents seems also to be mediated by the vomeronasal organ (Johns et al., 1978).

1.4. Scent marking in rats.

Norway rats have been frequently used in studies concerning olfactory communication. The remaining section of this chapter will deal with Norway rats: it will discuss first why *R. norvegicus* is a good species to investigate scent marking, and then the differences between laboratory and wild rats. The following two sections will explain the setting of the enclosures and the colonies used in this

thesis, discussing their implications for social and scent marking behaviour. Two additional sections will discuss what is known about the social (competitive and sexual) behaviour and feeding behaviour of the species. The last section will review literature on rat communication, especially the roles that scent marking plays in competitive and sexual communication.

1.4.1. Introduction.

The Norway or brown rat (*Rattus norvegicus* Berkenhout) along with the house mouse (*Mus musculus domesticus* Schwarze & Schwarze), and to a lesser extent, the roof rat (*Rattus rattus* L.), are the most successful mammals apart from humans (except the roof rat in Europe, see below). The reason for their success is that they are species which live commensally with us, at the expense of our civilisation. Brown rats are believed to have originated in Central Asia (Greaves, 1982). In the XVIII century they colonised Europe. Roof rats, earlier invaders also coming from Asia, were outcompeted by this temperate species and, by the end of the century, Norway rats had almost eliminated roof rats from Britain (Kowalski, 1976). Roof rats are still abundant in warmer European countries like Spain and Italy, and, surprisingly, they are considered one of the top ten endangered rodents in Europe (Lidicker, 1989).

Rats have adapted to human made environments and they can feed on most foods. They can jump 0.77 m from a stationary position and swim for 50-72 hours before exhausting themselves (Brooks and Rowe, 1979). Such abilities enable rats to feed in water on food destined for farmed fish and even to prey on them (Cottam, 1948). Some wild populations of rats in Italy dive for molluscs (Galef, 1980).

Despite their wide tastes in food, individual rats are very reluctant to eat any novel foods, a behaviour that is termed 'neophobia' (Barnett and Cowan, 1976; Domjan, 1977; Corey, 1978). This may have arisen as a consequence of continuous poisoning by man through many generations. Moreover, rats learn to sample foods and associate any sickness with the ingested food, which is henceforth avoided (Garcia, 1968).

Rats are ubiquitous and well adapted to a changing, man-made environment. Norway rats are good burrowers. This ability has probably facilitated their adaptation to live in highly variable, 3-dimensional, environments such as buildings, sewage pipes, farms and similar constructions: objects stored in buildings and farms may be moved every day, disrupting the chemical orientation system of the rats; the stream of water in a sewage pipe may increase frequently and wipe out all chemical cues; new objects appear almost everyday in human inhabited constructions. Norway rats are more likely found in the basement and lower storeys of a building than in the ceiling or upper storeys, unlike *Rattus rattus* (Telle, 1966). Nesting sites near food sources are preferred whenever possible, especially if they have some slope and are not completely flat (Robitaille and Bovet, 1976; Lore and Flannelly, 1978), and Calhoun (1962) has suggested that the distance between nesting and food areas is an indicator of dominance.

One of the reasons why Norway rats have adapted well to a commensal life with humans may be that they are nocturnal. Wild rats show a bimodal pattern of activity, increasing activity at sunset and sunrise (Barnett, 1975; Robitaille and Bovet, 1976; Takahashi and Lore, 1980; Nieder, 1985). Nieder (1985) found that rat activity was positively correlated with temperature.

Because rats are nocturnal and adapted to live in burrows and at ground level where vegetation intercepts a great proportion of incoming light, it is not surprising that they have poor sight. They are unable to see colour (Greaves, 1982), but can detect shape and movement in very dim light (Brooks, 1979; Greaves, 1982).

Their sense of touch, through their mystacial vibrissae seems to be very important in aiding orientation. Devibrissaed rats show a poor maze performance, drown in water, and cannot jump (Gustafson and Feilbain-Keramidas, 1977).

Rats have an acute sense of hearing. They can produce ultrasounds which they seem to use for sexual and aggressive communication (reviewed by Adler and Anisko, 1979 and section 1.4.7.1). They can use echoes of the sounds they make when moving, for echolocation during orientation in a maze (Riley and Rosenzweig, 1957).

However, their most developed sense seems to be olfaction. Slotnick, Kufera and Silberberg (1991) found astonishing odour learning abilities in laboratory rats. They presented the rats with sets of 8 non-social odours selected from 100 different chemicals. In each set, 4 were associated with a reward, whereas the remaining 4 were not. During the learning phase, 9 batches of 8 odours were presented twice in a random presentation. When rats were tested with 8 odours presented 5 times to assess retention, they showed 90-100% of correct responses after reaching stability in response, and 75-80% within the first 3-4 trials. Despite such abilities, some researchers have pointed out that '*the ability to smell does not seem to be an essential component of social attraction*' (Latané et al., 1972) and others have stated that '*the majority of works found in the literature mention odours as an important factor in the development of social lines*

and the unleashing of aggressive behaviour, but very few authors have studied more deeply the role these odours play' (García-Brull, Núñez and Núñez, 1993). However, the literature overwhelmingly indicates that odours seem to play a major role in rat social behaviour and possibly also in their orientation.

Rats scent mark their environment by depositing urine, by dragging their anogenital area on the ground or on objects, and also by rubbing their flank, a scent marking behaviour termed flank-marking, against the substratum, objects and burrow entrances (Grant and Mackintosh, 1963). In addition, rats also deposit urine on conspecifics (Grant and Mackintosh, 1963; Taylor et al., 1984; Taylor, Griffin and Rupich, 1988). Furthermore, as mentioned earlier, rats can use a number of olfactory cues from several sources to gather information about the diets of conspecifics (Galef, 1988), while lactating rats produce a pheromone in their faeces which attracts pups (Leon, 1974).

1.4.2. Why study scent marking in *Rattus norvegicus*.

The Norway rat constitutes a good model for working on scent marking and has been, along with mice, widely used in these kinds of studies.

Rats have been one of the main mammal models for all sorts of laboratory studies. This provides an extensive set of knowledge on the species, which allows us to relate scent marking to many other aspects of their biology, such as their social behaviour, learning abilities, physiology, biochemistry, etc. This is probably greater than for any other species of mammal, with the exception of mice (*Mus musculus domesticus*).

Rats are relatively easy to keep and habituate very well to captive environments, at least in the case of laboratory strains. Rats are quite small so that it is feasible to establish relatively large colonies in smaller areas than those required by larger mammals. In an urban area, Davis, Emlen and Stokes (1948) recaptured most wild rats within 25 m of their release point, although if food and shelter is separated, rats moved far more. Thus, it is possible to provide a naturalistic setting for a colony of rats in a relatively small space, similar to the conditions they would find in a city building or a stable, for example.

Rats are good candidates for research on scent marking and communication because they appear to rely heavily on scent cues, particularly those in urine, for a number of functions (reviewed by Brown, 1985b; section 1.4.6.2). Urine and other sources of scent can be obtained easily from rats in laboratory cages. Also, wild rats readily mark natural and man-made objects in their environment (Calhoun, 1962; Telle, 1966). This means that they can easily be tested using laboratory procedures, and thus, it is possible to study their natural behaviour in a man-made environment without being greatly stressed as would be the case in other wild mammals.

Finally, rats are interesting for their adaptability to a changing environment, which, as mentioned earlier, has made them suited to a commensal existence with humans. In our urban environment we produce the most rapidly changing habitat that any species could find. Rats adapt and thrive in our cities despite our efforts to eradicate them. Since odours potentially may play fundamental roles in their orientation and communication, understanding these roles is likely to be essential to both improve our existing systems for the control of rat populations and to design new ones.

1.4.3. Comparison of studies using wild versus laboratory rats.

Laboratory strains have been used in most studies dealing with rats, including behavioural studies. Laboratory rats are used on the assumption that their behaviour and performance is very similar or equal to that of their wild relatives. Although Boice (1981) has argued than laboratory rats are as well adapted to survive in the wild as wild rats, there seem to be some differences between them:

Wild rats seem to be more aggressive than laboratory rats. Wild male intruders have a high mortality rate when placed in enclosed, mixed sex colonies of wild rats (Barnett, 1958). In contrast, intruders in colonies of laboratory rats do not die (Barnett, 1975) or have low death rates (Barnett, Dickson and Hocking, 1979; death rate in intruders: 5% in laboratory males, 22% in wild males reared in laboratory, 61% in wild caught males). The cause of death in each case was the aggression of residents towards intruders. Both laboratory strains and wild rats reared in the laboratory are less territorial than wild-caught rats (Barnett and Cowan, 1976). This difference in aggression can be found even between wild-caught rats and the first generation of wild rats reared in the laboratory (Price, 1978). It appears that rearing or keeping rats in laboratory conditions reduces their aggressiveness. Laboratory rats, in addition, have been selected for their tameness.

As Adams and Boice (1983) found that dominance hierarchies among male laboratory rats of less than 150 days of age were not stable, it could be argued that perhaps laboratory rats had low death rates because the individuals used were young. However, laboratory rats were not found to have a high death rate even when the

individuals forming the colony were 46-50 weeks old (Barnett and Hocking, 1981).

Further evidence for differences in the aggressiveness of wild and laboratory rats comes from a study of weight and age as predictors of dominance. Berdoy, Smith and Macdonald (1995), have found that weight is an important predictor of dominance among laboratory littermates, but a bad predictor among wild laboratory-born non-littermates. In wild rats, age was a better predictor of status, and some wild rats dominated conspecifics larger than themselves.

The housing regime used appears to have a great influence on aggressiveness. Behavioural displays between rats become more aggressive as the degree of confinement is lessened (Boice and Adams, 1983). Thus, laboratory rats in large outdoor pens are more aggressive than those in smaller enclosures, which, in turn are less aggressive than rats in laboratory cages. Dominance only appears to arise in indoor/outdoor pens, not in laboratory cages (Adams, 1985). No similar study has been conducted in wild rats, where a number of additional variables may confound the effect of housing regime (such as greater stress when caged). However, there is evidence suggesting that aggressiveness may be very high in free-ranging rats: Calhoun (1948) found that only 16% of wild rats introduced in urban populations of free-ranging rats were recaptured. Although migration may have occurred, the ratio of intruders to residents found dead and the recapture data from neighbouring areas led Calhoun to presume that most of the missing intruders died, a death rates 3 times higher than that of residents. The high death rate in this case could be due partly to the greater aggressiveness of wild rats, although their unfamiliarity with the area

where they were released may have made them prone to accidents, predation, etc.

Isolation also seems to have a strong influence on aggressiveness. Blanchard and Blanchard (1980) found that rats living in colonies are more aggressive than isolated rats (but see Brain et al., 1980). Experience of fighting, and familiarity with their site of residence, were the most important factors regulating aggressiveness in Blanchard and Blanchard's study. In a series of tests using isolated versus colony reared laboratory rats, serious injuries, aggressive behaviour, gastric ulcers and weight loss only occurred where isolated intruders were introduced into colonies of rats that had been reared in groups (Luciano and Lore, 1975; Flannelly and Thor, 1976b; Brain et al., 1980). In contrast, other researchers found that isolation at an early age (in laboratory rats from 16 to 41 days of age) increased aggression whilst later isolation (from 41 to 68 days of age) had no effect on aggression (Wahlstrand, Knutson and Vike, 1983). Long term isolation (3-4 weeks in the same strain of rats) also seems to increase aggression (Wolffgramm, 1990). The rats used by Wolffgramm (1990) were young individuals (120-130 g at the start of the isolation period) of unspecified age. Isolated rats spend more time together than group-housed individuals when they are allowed to interact (Latané and Steele, 1975). Brain et al. (1980) showed that isolated males and males paired with females display more attacks than pairs of males. Brown (1991) found that rearing laboratory males in isolation also disrupts their preferences in odour investigation and urine marking while those with social experience of other males mark more than isolates, although he found no effect of isolation on female rats.

The existence of, or opportunity to dig, burrows further influences aggression in rats. Laboratory rats have a similar ability to dig burrows as wild rats (Boice, 1977). Blanchard, Blanchard and Flannelly (1985) found that male mortality and aggression was higher in colonies of laboratory rats with burrows that in colonies without them. The presence of burrows appears to accentuate dominance (Flannelly and Lore, 1977a).

Wild rats seem to be more excitable than laboratory strains. Laboratory rats are more active and defecate less in a novel environment than wild rats, although wild rats fight more (Harkins, Becker and Wright, 1974). The higher excitability, or fear found in such wild rats seems likely to derive from experiential effects such as trapping, habituating them to the laboratory for only a short period (14 days) and housing them singly, rather than from inherent differences between wild and laboratory rats. However, additional evidence for strain differences in excitability comes from the finding that wild rats and their laboratory-reared offspring are more defensive than laboratory rats, at least towards humans (Blanchard et al., 1986). Similar differences in excitability have been found between laboratory strains of rats and the offspring of crosses between laboratory and wild rats (Natynczuck, 1990). These differences do not seem to be attributable to early learning from a wild relative, as the offspring of a wild male rat reared by their laboratory mother alone appear to be more excitable, more active, and run faster than their mother (personal observation), despite the fact that the litter was reared in a laboratory cage.

The higher excitability of wild rats has been attributed to a bias in the status of the individuals captured by trapping (Boice, 1981). Traps are salient features of the home environment in the

field, and are avoided by rats. However, low-rank highly-excitable individuals may tend to be caught in them because they are displaced from familiar food sources and nests (Calhoun, 1962; Boice, 1981). Boice (1981) suggested trapping rats using the same procedure but doing it in a landfill. In such a highly variable and continuously changing environment, traps are not salient features and a better sample of the social structure of wild rats might be obtained. However some researchers have found higher excitability in wild rats compared to laboratory strains despite capturing the rats at a landfill (Harkins, Becker and Wright, 1974, but see earlier discussion of their results). Nevertheless, Boice (1977) was unable to see serious fighting in feralized laboratory rats, and found that the adrenal glands of laboratory rats were smaller than those of wild rats (Boice, 1981). This might be an indication of a higher degree of stress and excitability, in addition to greater aggressiveness in their wild captured rats.

Domestication seems to have altered the reproductive behaviour of rats. Although wild and laboratory rats do not differ qualitatively in the pattern of sexual behaviour shown (Dewsbury, 1975), wild rats have fewer intromissions per ejaculatory series, shorter ejaculatory latencies and longer ejaculatory clasps than laboratory rats. This is probably an adaptation to compete with other males (Price, 1980). As mentioned earlier, swarms of males may follow a female and rush to get a mating opportunity (Calhoun, 1962; Robitaille and Bovet, 1976). The process of laboratory isolation accentuates the slowing down of the copulatory sequence (Thor, 1980). Domestication has also reduced the dependence of breeding upon the photoperiod (Shishkina and Borodin, 1986).

Wild rats are more neophobic than laboratory rats (Barnett and Cowan, 1976). This is likely to have arisen from the constant poisoning threat wild rats face when sampling new foods in their natural environment. Neophobia seems to be greater also in the laboratory-born offspring of wild rats. Despite the greater aggressiveness of wild rats and their offspring, Price, Belanger and Duncan (1976) found that male laboratory rats become dominant to male laboratory-born wild rats when competing for a novel food in a laboratory cage.

Scent marking and investigation differences. It seems difficult to discern from the literature whether differences found in scent marking and investigation between wild and laboratory rats are intrinsic or can be attributed to housing regime, experimental design, or other such factors. The most obvious difference concerns flank marking. Calhoun (1962) reported that wild males frequently rubbed against stones, burrow entrances, or places where females had recently marked. However, only one report appears to have recorded flank marking in laboratory rats, that of Peden and Timberlake (1990). This might be due to a truly more frequent marking by wild rats compared to laboratory strains. However, this aspect remains to be addressed.

In conclusion: There are major differences in aggressiveness and other aspects of behaviour between wild and laboratory rats. Although wild rats are much more difficult to handle, they constitute a better archetype for the species as a whole and, thus, in studies addressing questions of relevance to rats per se, wild rats should be used whenever possible.

The housing regime also has a great influence on social behaviour. Rats reared in groups seem to be more representative of

natural social conditions than those reared in isolation. Likewise, mixed-sex colonies of rats constitute a better model than isolates (but see Lore, Nikoletseas and Flannelly, 1980). In a similar way, laboratory cages disrupt social behaviour, and large pens with burrowing facilities constitute a better alternative. The ideal situation for working with wild rats thus would appear to be a field study of wild ranging rats (similar to Ewer, 1971 in *Rattus rattus* or Robitaille and Bovet, 1976 in *Rattus norvegicus*). However, because individuals marked for identification may migrate, because in order to avoid disturbance their home range or individual experience frequently cannot be manipulated and because observation under such free-range conditions is extremely difficult if not impossible, studies under such conditions are limited largely to descriptive rather than experimental research. Similarly, large outdoor pens also allow little control of experimental conditions, and in small room laboratory enclosures the population density soon becomes too high (Calhoun, 1962).

The best compromise for a research on rat behaviour aiming to perform experimentation and observation seems to be large indoor enclosures with burrowing facilities housing wild trapped rats (Shepherd and Inglis, 1987, see section 2.1 for a thorough discussion). This is the approach used in the experiments reported in this thesis.

1.4.4. The setting of the experiments.

The following two sections will explain the setting and strain of rat used, relating these to the factors affecting social behaviour of rats, and the differences between wild and laboratory rats.

1.4.4.1. The enclosures.

As mentioned in the previous section, large enclosures seem to be the best compromise between the necessity for controlling variables and that of keeping rats in an environment as similar as possible to their natural home range. The enclosures used in my experiments provided a similar environment to that found by rats in farm buildings. Temperature, humidity and light were not constant but depended on weather conditions. However, despite these natural conditions, a captive regime is likely to influence behaviour, compared to that of free-ranging rats. Rats released from the threat of predators, as they were in the enclosures, and with easy access to food and water ad libitum may divert a greater part of their energies and time into patrolling their territory and to maintaining their rank in the hierarchy. Although social interactions in captive colonies, especially aggression and territorial behaviour, may be stronger than those in free-ranging rats, early research in free-ranging (Calhoun, 1948; Davis, Emlen and Stokes, 1948) and captive colonies of wild rats (Barnett, 1958; Calhoun, 1962), showed similar death rates due to aggression in both. Thus, the hypothesis that captive regimes, or at least those where rats are kept in large enclosures, have a very significant influence on aggressive behaviour compared to free-ranging regimes seems to receive little support from the literature.

1.4.4.2. The colonies.

There are three possibilities for the social setting of rat pens: to keep isolated rats, to form single sex colonies, or to form mixed sex colonies of rats. As discussed earlier, there is controversy over the effect of isolation on aggression.

A mixed sex colony seems a better alternative than a single sex one. Mixed sex colonies appear to show high levels of aggression compared to single sex colonies. Males living with females, not those living with males, display increased levels of aggression (Flannelly and Lore, 1977a). Similarly, wild rats in larger colonies consisting of individuals of both sexes display more aggression towards intruders than colonies consisting only of males (Barnett, 1958).

Thus, a mixed sex colony seemed the best compromise for the experiments reported here. One of the eight colonies used in the experiments was a large colony consisting of 17 rats, the others consisted of pairs of rats. Pairs of rats were used: i) to reduce the number of confounding variables, such as the unknown influence of juveniles on the experiments. Although the contribution of juveniles to scent marking and their responses to the stimuli of the tests seemed interesting to study, their influence may have obscured the responses of dominant residents towards intruders which were the main goal of the study; ii) using pairs of rats eliminated the confounding effect of the female aggression due to the onset increased aggression in lactating females; iii) as odours are likely to play a role in competitive and sexual behaviour (see section 1.4.7.2.5 and 1.4.7.2.6), which vary with the season, it was preferable to conduct the experiments during the season of highest activity of the rats, i.e. the summer. In such a short experimental period, colonies formed by pairs of rats constituted a good compromise to be able to replicate experiments in two sets of colonies per year; v) from an ethical point of view, using pairs of rats had the advantage of reducing to a minimum the number of rats to dispose of after the

experiments (ASAB Guidelines for the use of animals in research, 1995), which I found the hardest part of the research procedure.

Pairs of rats are referred to in this thesis as colonies. Although interactions between individuals of the same sex and parental behaviour do not occur in such colonies, they display other typical aggressive and sexual behaviours compared to dyads of males, as pointed out above. In addition, a pair of rats seems a naturalistic setting because this is likely to be the case when rats colonise new areas. Information regarding the size of free-ranging colonies of rats in the literature is very scarce and highly variable. Telle (1966) found that rats in colonies under 20 individuals rats are likely to defend their territory. However, in groups larger than 20 individuals (telle (1966) reported groups larger than 80 rats), territory defence and individual recognition seems to fail. Robitaille and Bovet (1976) studied rats in a rubbish landfill where population density reached 1-2 rats/m^2, which seems to be unrepresentatively high. It should be noted that small colonies, especially colonising pairs, are likely to be under-represented in any such studies because of the difficulty in finding and observing them.

1.4.5. Social behaviour in *Rattus norvegicus*.

1.4.5.1. Competitive behaviour.

Before discussing aggression, dominance and territoriality in rats, it is worthwhile discussing the existing systems in competitive behaviour in mammals and their alternative definitions. These have been shaped by evolution and, in some cases, the same species in different environments will adopt a different social organisation (Monaghan, 1990).

Individuals may occupy an **undefended home range.** However, in some cases home ranges do not overlap as a result of behavioural exclusion of conspecifics or territoriality.

Definitions of territoriality can be classified as either ecological or behavioural (Maher and Lott, 1995). Ecological definitions are concerned with the distribution of animals in space, whereas behavioural definitions are concerned with interactions between individuals. These differences can be of great importance when discussing whether rats are territorial or not. Most of the literature on rats has been produced by psychologists and, hence, deals with social interactions rather than with the distribution of rats in the wild (as opposed to early research on rats, reviewed by Davis, 1987).

Maher and Lott (1995) found three definitions of territory used in the literature:

-A defended area (behavioural definition). "Defended" usually means aggressive behaviours towards intruders like chasing, fighting, etc. and advertisement by the owner, including scent marking (Brown and Orians, 1970).

-**An area of exclusive use** by the owner or where other individuals are excluded (ecological definition). Brown and Orians (1970) have argued that this type of definition, usually inferred from non-overlapping home ranges of individuals, may cause confusion because the reason for this lack of overlapping may not be behavioural. Thus, it should not be used unless accompanied by observations of exclusion of conspecifics.

-**An area where an individual is dominant over its conspecifics** and where it has priority of access to resources (behavioural definition). This definition is site-specific: An

individual A dominant to individual B in A's territory, will not be dominant to B in B's territory.

An alternative competitive social system (Maher and Lott, 1995) is a dominance hierarchy, based on aggression, of some individuals over others, where the outcome of fights can be reliably predicted but is not site-specific.

Gray and Hurst (in press) have suggested that dominance hierarchies arise when the habitat is too complex for the owner to successfully exclude conspecifics. Ens, Weissing and Drent (1995) suggest that, when the habitat is saturated (independently of its complexity), individuals may have to establish site-specific dominance in order to overcome the territory owner's advantage. Familiarity with the site may be important in fights and, thus, newcomers would not fight frequently because they might have to win more than once (and occupy a high rank in the hierarchy) to hold the territory.

Brown and Orians (1970) indicated that individuals may defend a territory when: i) this would reduce predation and parasite risk; ii) resources (food, females, etc.) are economically defensible; and iii) a territory rich in resources attracts more females. When resources are very abundant, very scattered, or competition pressure very high it would not pay individuals to defend these resources. In the following paragraphs I shall discuss the type of social system shown by rats under different circumstances and then I will review the benefits that the dominant individual/territory owner may obtain.

Type of social system. Rats do not seem to occupy an undefended home range. Researchers in early experiments found that almost all rats introduced into an already established free-

ranging urban population were recovered within 25 m of their original release point (Davis, Emlen and Stokes, 1948) and none of them left the urban block where they had been released (Calhoun, 1948). These introduced rats seem to have been prevented from entering other territories and to have sustained prolonged aggression, as their death rate was three times that of the residents (Calhoun, 1948). However, social systems may not be rigid and, as pointed out earlier, if resources are very scattered, territoriality may not compensate its costs. Thus, if food and shelter are distant from each other, rats may travel several kilometres in one night (Davis, Emlen and Stokes, 1948; Telle, 1966). It is not clear whether these wandering rats were venturing into territories occupied by other rats and, if they were doing so, whether they were allowed to pass through or were chased out.

Calhoun (1962) found that rats kept in a large open enclosure (924 m^2) excluded others from the area that they occupied. Territoriality, following the ecological definition of an area of exclusive use, was very common among lactating females. Mothers drove off intruders from their nesting area, but did not show any sign of aggression elsewhere, even towards the same individuals previously attacked. He also observed that males excluded other males whenever they could defend a harem (i.e., when females were economically defensible). Telle (1966) observed exclusion of intruders also in a free-ranging situation. In this case, rats defended trails that they marked with urine and used as pathways to move around. They also defended the immediate vicinity of the trails and burrow entrances.

However, Robitaille and Bovet (1976), working on wild free-ranging rats, failed to find exclusion of intruders. Again, the

distribution of resources may have made defence of the territory unsuitable or impossible. The population of rats studied lived in a rubbish landfill and had a large amount of food available. As a result, the population density was very high (2-3 rats/m^2). Both frequency of social interactions and resource density were very high. Under such conditions, territories may not be economically defensible as defined by Brown and Orians (1970). Furthermore, Telle (1966) reported that in wild rats living in large groups (more than 20 individuals), aggression was markedly reduced and intruders were accepted. He suggested that in such high population densities, individual recognition may fail and, as a consequence, aggressiveness would be reduced. Hence, the high population density in Robitaille and Bovet's study may account for the lack of territorial exclusion observed, although Robitaille and Bovet did find a hierarchy despite the high population density (see below).

Both wild and laboratory rats kept in enclosures seem always to behave aggressively towards intruders (Barnett, 1958, 1975). In such a situation, where space is very limited (section 2.1), no escape is possible since intruders and residents cannot leave their home range. On the one hand it is impossible to find territorial exclusion (the ecological definition of territoriality). On the other hand, it is not possible to compare the interaction of a pair of individuals within and outside a defended territory and thus discriminate between site-specific dominance (a definition of territoriality) and dominance per se. Most studies on aggression, hence, cannot discriminate between these two social systems.

Although Telle (1966) failed to find a social hierarchy in wild free-ranging rats, many other researchers have found some kind of dominance in wild or laboratory rats, either free-ranging or in

captive colonies. Dominance hierarchies are usually defined by the outcome of aggression (Grant, 1963), or priority of access to females (Adams and Boice, 1983). Hierarchies can also be discerned from inequalities in two other behaviours which correlate with hierarchies based on aggression outcomes: passing, where the dominant rat overtakes the subordinate one, and crawling over, where the dominant individual is the one crawling over the other rat (Ziporyn and McClintock, 1991).

Although most literature on dominance deals with male dominance, hierarchies have also been found in females (Adams and Boice, 1983; Ziporyn and McClintock, 1991).

Male dominance. Dominance occurs in laboratory rats. Individual adult males have been found to be consistently dominant (defined as outcome of fights) over a long period of time (Adams and Boice, 1983). Blanchard et al. (1988b) found that dominance hierarchies were very stable over the life span of rats. Hierarchies were found to appear at 140 days of age by Adams and Boice (1989). They found that, in males younger than 150 days of age, hierarchies were neither stable nor functional (Adams and Boice, 1983). Blanchard et al. (1988a) argue that dominance is consistent because, as they found, individuals that are highly aggressive when they first meet other colony members later become dominant. This study also showed that, as the dominance rank was established, the level of aggression decreased.

Dominance has also been found in wild rats. A near linear pattern of dominance among males has been reported in colonies of wild rats living in large outdoor enclosures, both in single sex and mixed sex colonies (Berdoy, Smith, and Macdonald, 1995; Berdoy, Webster and Macdonald, 1995). Berdoy, Smith and Macdonald

(1995) found that the outcome of the first fight greatly influences the outcome of subsequent fights. In a study of wild free-ranging rats, Robitaille and Bovet (1976) observed that 94% of aggressive encounters involved individuals of the same size class in their study. Since males never defeat conspecifics much larger than themselves (Barnett, 1958), the largest male was accustomed to be avoided by other rats and thus was never observed to be challenged by other males (Robitaille and Bovet, 1976). Barnett (1958), on the contrary, found that the dominant or alpha male in captive colonies of wild rats always behaves aggressively to other rats. Juveniles are never attacked (wild free-ranging rats: Robitaille and Bovet, 1976; laboratory rats: Thor, 1979), even when they are intruders, as long as they are sexually immature (Thor and Flannelly, 1976a; Flannelly and Thor, 1978). Thus, it is possible that juveniles are allowed into new territories where the dominant male habituates to and tolerates them as they mature (Thor, 1979). Males, especially the dominant, even attack females, although these attacks are less frequent and aggressive than towards mature males (Barnett, 1958; Calhoun, 1962; Alberts and Galef, 1973; Thor and Flannelly, 1976b; Barnett, Dickson and Hocking, 1979). Besides dominant males and females, subordinates and juveniles, there is a fourth social rank of displaced individuals (what Calhoun (1962) called "social outcasts"), consisting of very shy males and females which always lose fights and avoid conspecifics (Barnett, 1958; Calhoun, 1962). They show diurnality, slow growth, low adult weight and a marked tendency to re-enter traps (Calhoun, 1962). Although it has been reported that socially displaced individuals are immigrants from other rat groups (Barnett, 1958), individuals with such characteristics have been seen within a large wild colony of rats (17 members) kept in a 50 m^2 enclosure

where emigration was impossible (author, personal observation). These socially displaced individuals might be individuals excluded from nesting and feeding areas (perhaps the reason for their diurnal activity), that cannot migrate as a result of being kept in enclosures. In aggreement with this, Hurst et al. (1996), working with laboratory strains, have found that low status individuals sleep less, spend more time trying to escape and exhibit a number of negative pathophysiological symptoms (see below).

In conclusion, there appears to be a linear dominance hierarchy within rat groups but, in most cases, tests have not been carried out to discern whether this is site-specific. Calhoun (1962), however, found that males did not fight outside their defended territory, which suggests that the rat dominance system is site specific, at least in wild rats.

Intruders are attacked by colony members. The dominant male is responsible for most of this attack behaviour (60-80% in Blanchard, R.J. et al., 1984). While some researchers have reported that subordinates behave in a subordinate way *to intruders* (Barnett, 1958), others have reported that all colony members contribute to attacks (Blanchard et al., 1975; Adams and Boice, 1989). The aggression of dominant males and females is directed mainly towards intruders of their own sex (Brain et al., 1980; Blanchard, D.C., et al., 1984). Intruders placed into captive colonies of wild rats have a high death rate, but introductions do not result in casualties in colonies of laboratory rat strains (Barnett, 1975). Intruders not only survive in inescapable captive situations, some intruders in wild free-ranging rats are incorporated successfully into rat colonies (Calhoun, 1948; Telle, 1966).

Factors influencing dominance in male rats. A number of factors have been found to influence aggression and dominance among male rats: age, fighting experience, and the presence of females, among others.

Age is a better predictor of dominance than weight in wild rats (Berdoy, Smith and Macdonald, 1995; Berdoy, Webster and Macdonald, 1995). In laboratory rats, Blanchard et al. (1988a) also found that some aggressive dominant males weighed less than subordinate males, although Hurst et al. (1996) found opposite results in age-matched rats.

Although some researchers have found that experience in resident-intruder fights has little influence on dominance (Adams, 1985), general experience in fighting does seem to affect aggressiveness. Offensive behaviour may be somewhat diminished by experience of defeat (Adams, 1985), whereas experience of victory increases subsequent fighting and victory (Blanchard et al., 1977a; Kemble et al., 1985). Rats seem to establish dominance not on the basis of overall fighting experience but on the outcome of previous encounters with each individual rat (Berdoy, Smith and Macdonald, 1995). Aggression, on the other hand, is higher when the individual is tested in its home cage (Mink and Adams, 1981).

The presence of females increases male aggression towards intruders. Thus, intruder males suffer high mortality in colonies that include wild rats of both sexes, but low mortality in colonies consisting only of wild males (Barnett, 1958). Similarly, laboratory males living with females are more aggressive towards intruders than those living with other males (Flannelly and Lore, 1977b). The effect could be due to sexual activities, as Flannelly et al. (1982) found males allowed to copulate up to ejaculation showed increased

attack towards unfamiliar males compared to males interacting with anoestrus females, with inaccessible oestrus females or with no females. Taylor, Weiss and Rupich (1987), examined the effect of female presence on male aggressiveness and physiology. Males were exposed either to groups of aggressive unfamiliar males where individuals were constantly renewed (high stress settings) or to less aggressive and familiar groups of males (low stress setting). Taylor, Weiss and Rupich (1987) found that the presence of females increased male aggressiveness in both high and low stress settings, but also reduced the consequences of stress for those males in high stress settings, resulting in smaller adrenal weight and lower levels of corticosterone than among males in similar high stress groups without females.

Benefits for the dominant male. Dominance in rats does not seem to result in exclusive access to resources. However, dominant males may gain preferential access to females and food, result in greater growth rates and reduce the physiological effects of stress.

Male aggression and dominance seems to establish priority of access to females and, less markedly, to food (Blanchard, D.C. et al., 1984). Although dominant males do not show greater sexual activity than subordinates (Brown, 1974), when tested separately in choice tests, females visited more, spent more time and mated more with the dominant than with the subordinate male (Carr et al., 1982). Similarly, in a free competition test between 2 males and 1 female, the mean number of ejaculations by the dominant male was higher than that of the subordinate (Thor & Carr, 1979). Dewsbury and Hartung (1980) found that the male ejaculating last sires most offspring. Flannelly and Lore (1977a) found that only the

dominant male mounted females after their first lordosis. McClintock, Anisko and Adler (1982) found that, in a multiple mating situation, where 2 males were presented with four females in oestrous, females received more ejaculations from the dominant male. They also competed for his sperm and rested longer after receiving an ejaculation from the dominant male, which would be likely to favour the transfer of the dominant male's sperm. In a large outdoor enclosure housing a colony of wild rats, Berdoy, Webster and Macdonald (1995) found that the dominant individual had the highest reproductive success: he participated in the greatest proportion of chases of females during a swarming of males, had the highest proportion of copulation attempts, and the highest proportion of ejaculations. However, there was no overall difference among other individuals, despite the linear hierarchy among them.

No fighting for females has been observed between albino males in laboratory studies (Dewsbury and Hartung, 1980; McClintock, Anisko and Adler, 1982). A similar lack of aggression has been reported in wild free-ranging rats (Telle, 1966), but sometimes males may fight over counter-marking a female's marks and, if only a couple of males are trying to mate with her, they may fight to mount her (Calhoun, 1962; Robitaille and Bovet, 1976). Robitaille and Bovet (1976) found stronger evidence concerning the advantage that a dominant male has when mating. They observed that sometimes the largest male guarded the burrow where an oestrous female was, precluding other males from approaching her and forcing the female to stay inside. The female was only allowed to emerge to mate, and then was forced back into the burrow. In this case, dominance ensured exclusive access to a receptive female and thus, paternity of her offspring.

Dominance may improve access to food. Although no fighting has been observed during communal feeding at food hoppers in wild rats (Calhoun, 1962; author, personal observations), Smith, Smith and Sibly (1991) found that dominant males may spend more time than subordinates feeding from a single food source. This suggests that dominants exclude subordinates from the feeder, or conversely, that subordinates avoid the dominant. In a choice test between a preferred and a non-preferred food, Smith, Smith and Sibly (1991) found that dominants exclude subordinates without overt aggression. Thullier et al. (1992) observed two types of individuals: rats which usually stayed in the feeder to eat (which they called 'non-carrier rats'), and rats who took food and carried it away from the feeder to eat (which they called 'carrier rats'). They reported that in fights for food, most food was stolen by 'non-carrier' rats from 'carrier rats'. Although these researchers did not make explicit mention of dominant or subordinate rats, the results suggest that rats stealing food (non-carrier rats) were dominant over those that carried food away. Calhoun (1962) found that the proximity of a burrow to food sources was an indicator of dominance in wild rats.

Dominance seems to influence growth. In short term studies (a few weeks at most), dominants either gained weight whereas subordinates or intruders lost it (Van de Poll et al., 1982; Raab et al., 1986), or they gained more weight than subordinates (Flannelly and Lore, 1975; Williams and Lierle, 1988; Hurst et al., 1996). As pointed out earlier, Calhoun (1962) observed that socially displaced individuals grew slowly compared to other colony members. In a two year study of wild rats kept in a large enclosure, Berdoy, Webster and Macdonald (1995) found that dominance was not

correlated with long term rate of growth. However, there was a confounding factor in this study: younger individuals were taken into consideration along with older mature males. Hence, growth was greatest for juvenile individuals who had low social rank.

Dominance also affects physiology. Barnett, Dickson and Hocking (1979) found that omega males (socially displaced individuals), may have hypertrophied adrenals, which could be caused by high stress. Subordinates have heavier adrenals than controls or dominants (Davis 1987; Blanchard et al., 1993), higher titres of corticosteroids (Blanchard and Blanchard, 1990; Blanchard et al, 1993), reduced immuno-competence as measured by reduced lymphocyte response (Raab et al., 1986) and antibodies (Blanchard and Blanchard, 1990), and greater risk of infection by *Trichinella* (Davis, 1987). Hurst et al. (1996) have found that kidney weight relative to body weight and signs of early kidney pathology were greater in individuals of low social rank, although no adrenal congestion, or changes in thymus or immunoglobulin G titres were found. They also found that an overall arbitrary score of pathology in adrenal, kidney, thymus and testis tissues was greater the less time that individual rats spent sleeping (a time behaviour which occupied a lower proportion of the time budget of low status individuals, see above). In contrast, corticosterone levels were higher in males that attempted to attain dominant status but failed, whereas levels were lower in males that became dominants or did not attempt to compete (low status individuals). Similar differences are seen in the blood pressure and catecholamines of unsuccessful competitive males (Koolhaas et al., 1986).

Another physiological correlate of dominance is testis weight. Testes are lighter in subordinates than in dominants (Blanchard et

al., 1993). Testes are the primary centre for the production of testosterone, and this difference in weight may explain the lower testosterone levels found by Blanchard et al. (1993) in subordinates. Since dominance is correlated with aggression, a relationship between testosterone and aggression should be expected. Thus, Albert, Petrovic and Walsh (1989b) found that, other factors being equal, males injected with testosterone were more aggressive than sham injected ones. Testosterone also influences the aggression elicited by conspecifics. Hence, aggression is only directed towards intact males with normal testosterone levels, not towards females, castrates or prepubertal juveniles (Thor and Flannelly, 1976b). However, in one study of captive wild rats (Berdoy, Webster and Macdonald, 1995), and another of laboratory strains (Hurst et al., 1996), where male rats had unmanipulated (thus presumably normal) testosterone levels, testosterone titres were not correlated with dominance status.

Female dominance. Although little attention has been paid to female dominance and aggression, Ziporyn and McClintock (1991) found that females were as likely to fight as males. Some studies have found female dominance to be unstable and present only during pregnancy and lactation (Adams and Boice, 1983). Ziporyn and McClintock (1991) maintain that it is possible to discern a stable female dominance hierarchy which is not related to pregnancy on the basis of non-aggressive behaviours (crawling over and passing). Blanchard, D.C. et al. (1984) also found that female aggression was not related to lactation. In aggression tests where all colony individuals of the same sex were tested against male intruders, they observed that the dominant female was responsible for most of the attacks on intruders. Females also have been

observed to defend the territory in *Rattus rattus* (Ewer, 1971). Hurst et al. (1996) found that dominance hierarchies among females housed in single sex groups remained stable over an 8 week study period and that the pattern of aggression among was different from that among males: aggression was shown much more frequently by dominant than by subordinate females, involved much chasing and mounting and levels remained stable over time. In contrast, male aggression involved much more static posturing and, although initially much higher than in female groups, aggression declined over the 8 week study among all males.

Cohabitation with an intact male triggers the development of aggression in females prior to parturition (Albert et al., 1988). This 'maternal aggression' has also been observed in wild rats (Calhoun, 1962), where females actively excluded conspecifics from the areas close to their burrow (ecological definition of territoriality). Aggression peaks on the day of parturition and on day 9 of lactation (Flannelly, Flannelly and Lore, 1986), although the second peak was not found in a subsequent study (Flannelly and Flannelly, 1987). Aggression in laboratory rats was found to be greatest towards intact males, lower towards castrated males, and lowest towards females (Albert et al., 1988). Females also show aggression during pseudopregnancy, which continues at least up to day 13 after pseudopregnancy is over (Albert et al., 1991).

In contrast to aggressive situations involving two males, males often respond to female attack with aggression (Blanchard, D.C. et al., 1984; Flannelly, Flannelly and Lore, 1986). However, female aggression towards males and retaliation depends on the size of the male. Only 30% of females tested with larger intruder males (which were also older) attacked them, whereas 80% of females tested with

smaller intruder males (also younger) attacked them (Flannelly and Flannelly, 1985). In this study, however, no male retaliation was observed. Female aggression seems to be less serious than male aggression because females rarely produce wounds (Blanchard, D.C., et al., 1984). Male aggression against intruders, in contrast, usually produces wounds in the opponent (Blanchard et al., 1977b).

Factors affecting female dominance. Maternal aggression, like aggression in males, is hormone dependent (Mayer and Rosenblatt, 1987). Albert et al. (1989a) found a sharp drop in aggression when females were ovariectomised. Following ovariectomy, only hormone restoration could reduce or prevent the decline in aggression, depending on treatment (Albert, Jonik and Walsh, 1990, 1991).

Benefits for the dominant female. Dominant females seem to gain a number of benefits from their status: preference in breeding, reduced effects of stress, increased offspring survival, and probably other benefits that have not yet been studied.

Maternal aggression could serve to reduce social stress induced by interactions with other rats and increase survival probability for the offspring by excluding conspecifics from nesting areas. Social stress (as indicated by increased interactions with unfamiliar rats) results in aggression and immediate and complete cessation of litter production (Lobb and McCain, 1978). Since infanticide is greatly inhibited when a pregnant female is present (Brown, 1986a), maternal aggression may also serve to reduce infanticide by males. A by-product of female aggressiveness appears to be to reduce care of the offspring by the male (Brown, 1986b).

Dominance in females is related to the possibility of breeding. In mixed sex colonies of both laboratory and wild rats, Zyporin and

McClintock (1991) found that the dominant female was the first to become pregnant. This study also found that female dominance was not related to weight.

Changes in physiology. Hurst et al. (1996) found that negative pathophysiological changes in rats (a combination of kidney tissue changes and relative weight, adrenal congestion, thymic parameters, antibodies and corticosteron levels) were greater among individuals that suffered the greatest social pressure and frustration. In the case of females, these were low status individuals that were frequently attacked and attempted to escape from their enclosure-housed groups.

As there is little fighting between the sexes, other behavioural patterns related to dominance, like crawling over, may be used to discern the hierarchy between males and females (Zyporin and McClintock, 1991).

1.4.5.2. Sexual and reproductive behaviour.

The pattern of copulatory behaviour in rodents has been shown to be adapted to their habitat and life history (Dewsbury, 1975). In the case of rats this suggests that a locking mechanism in copulation has not evolved possibly because rats are adapted to a number of environments, including some man-made ones which do not give them enough tranquility and security for this mechanism to be safe (like sewages and areas where they cannot dig burrows). Dewsbury (1975) also pointed out that ejaculation on first insertion has only evolved in species living without any shelter, where longer copulation may greatly increase the risk of predation. Rats may not have evolved such a type of ejaculation presumably because they can dig burrows (Calhoun, 1962; Lore and Flannelly, 1978) or use

available shelter. Finally as rats are not a monogamous, pair-bonding species, they have not evolved the prolongued intromission latency periods that are common in such species (Dewsbury, 1975). The sequence of copulatory behaviours in rats has been described by Dewsbury (1967).

The typical pattern of mating observed in wild, free-ranging rats is somewhat different. The female travels when she is receptive (Calhoun, 1962), as in laboratory studies (Martin and Bätig, 1980). A swarm of several males may follow her (Calhoun, 1962; Telle, 1966; Robitaille and Bovet, 1976; Berdoy, Webster and Macdonald, 1995). Fighting is not usually seen in such multi-male/single female situations (Calhoun, 1962; Robitaille and Bovet, 1976), probably because a single male is unable to exclude many males and would lose opportunities to mate to other competitors. However, if only a few males (two, three) are present, they may fight to counter-mark the female's scent marks (Calhoun, 1962) or for the opportunity to mate if they are following the receptive female (Robitaille and Bovet, 1976). The largest male may guard a female in its burrow (section 1.4.5.1).

McClintock and Anisko (1982) have suggested that rats mate in a multi-male, multi-female pattern. This pattern would allow each sex to achieve successful reproduction through its particular strategy. Thus, females would benefit by increasing their rate of impregnation rates as a result of increased stimulation (McClintock, Anisko and Adler, 1982), whereas males would produce more ejaculatory series (McClintock and Anisko, 1982). However, this multi-female pattern has not been observed in the wild and does not seem to be the most likely situation. No fighting to mate with a female was observed either in this study, or in a 2 males/1 female

situation in the laboratory (Dewsbury and Hartung, 1980; see section1.4.5.1). However, the dominant male may increase its fertilisation by a number of means (section 1.4.5.1). Dewsbury and Hartung (1980) suggested that, as little fighting takes place in a 2 male: 1 female laboratory situation, the pattern of multiple ejaculations in rats might be an adaptation to sperm competition. Thus, the mating strategy of rats may swing from fighting to exclude other males, if this could ensure them sole access to the female, to a sperm competition strategy when females are not economically defensible.

Rats of both sexes seem to exhibit some kind of mate choice. Dominant females are more attractive to males (Adams and Boice, 1983) and, in captive colonies of one female and 5 male laboratory rats, the dominant male is responsible for 40% of the total frequency of anogenital investigation of the female (Flannelly and Lore, 1977a). While some researchers have found that partner novelty generally leads to more mutual approach than between familiar animals (Barefoot, Aspey and Olson, 1975), others have found this to be true only for males (Carr, Krames and Costanzo, 1970; Carr Hirsch and Balazs, 1980), whilst females either show no preference (Carr, Krames and Costanzo, 1970) or prefer the familiar male (Carr et al., 1979; French, Fitzpatrick and Law, 1972). French, Fitzpatrick and Law (1972) found that females in oestrus, but not in dioestrus, show a preference for some males over others. The preference of laboratory males for unfamiliar females might be an artefact of testing male rats with little sexual experience in an unfamiliar arena, and it might not occur in sexually-experienced wild males defending their territory. Although females seem to prefer dominant males (Carr et al., 1982; McClintock, Anisko and Adler, 1982), Taylor

and Weiss (1987) found that those mating with preferred males apparently did not have higher reproductive success than females mating with non-preferred mates. Unfortunately, no test was carried out in this latter study on dominance within the dyads of males.

Dyads of female laboratory rats rear offspring communally (Mennella et al., 1990). Mennella et al. (1990) found that litters born less than 14 days apart benefit from communal nursing, but if born more than 14 days apart the older litter is detrimental to the younger litter. Schultz and Lore (1993) found that families living together reared more pups to weaning age than isolated families. However, as mentioned earlier, studies on wild rats have found that lactating females actively exclude conspecifics from their nesting areas (Calhoun, 1962), and others have observed that wild free-ranging rats do not raise the young communally (Telle, 1966).

Although the past two sections show that sexual and competitive social behaviours are very important for rats, this does not necessarily mean that rats engage in both for similar proportions of time. Even in laboratory studies using previously isolated rats in a sexually receptive state, individuals occupy only 10% of their time in sex-related behaviours (Sloan and Latané, 1974). In a rat colony consisting of freely interacting individuals of both sexes, most adult females are likely to be either pregnant or lactating. If they enter another territory it seems more likely that they will constitute a potential competitor even for adult males (either competing for food, or because of the possibility that they will attack the male's offspring) rather than a potential mate.

1.4.6. Feeding behaviour.

In addition to the role that scents may play in rat social behaviour, their olfactory cues may affect their feeding behaviour. This section will discuss both rat feeding behaviour and the roles that olfactory cues have been suggested to play in this.

Rats are very eclectic and will eat almost anything, sampling all kinds of food that they find in their home range (Barnett, 1975; Barnett et al., 1978). The most salient feature of rat feeding behaviour is their neophobia. The term neophobia describes the habit of avoiding new objects. Rats show a strong neophobia towards new food items (reviewed by Domjan, 1977). This allows rats to avoid poisoning by humans. Thus, *Rattus norvegicus* and other commensal species such as *R. rattus* and *Mus musculus domesticus* are very neophobic towards both foods and new objects, whereas non-commensal species of rat such as *R. villosissimus* and *R. fuscipes* are not neophobic (Barnett and Cowan, 1976; Cowan, 1977).

Neophobia towards novel food containers in wild *R. norvegicus* is stronger than food neophobia (Shepherd and Inglis, 1987). Wild rats are more neophobic than laboratory rats (Mitchell, 1976). Differences are also found with respect to social rank and age. As dominants have better access to familiar foods, they are more reluctant to exploit new food sources, and hence they are more neophobic (Robertson, 1982). Adults are more neophobic than juveniles (Corey 1978; Shepherd and Inglis, 1987). Barnett (1975) reports the opposite effect, neophilia, for gnawing and exploring new areas (Barnett and Cowan, 1976). Wallace (1988), however, reports that rats are neophobic when exploring new areas. In this case, the new 'area' was a narrow alley maze. As alley mazes are

very similar to devices like traps, neophobia may have been found in this study as a result of human-induced selection on rats to avoid traps. Calhoun (1962) also reports that rats were very reluctant to enter similar devices (the alleys were automatic devices to count rats). Neophobia might not be found when rats are exploring new open areas, such as those used by Barnett and Cowan (1976). Some researchers found, however, that rats placed in a novel area have a strong motivation to return home. Thus, the hyperactivity a rat experiences when in a new open area is due to neophobia (because it tries to escape from that area) and not due to exploration (Blanchard, Kelley and Blanchard, 1974).

Another consequence of poisoning has been the evolution of resistance to poisons like the anti-coagulant warfarin. It was presumed that resistance to poisons had a cost of slowing growth (Smith, Townsend and Smith, 1991). Surprisingly however, in the absence of poison, warfarin-resistant individuals not only do not pay a cost of slower growth but they are actually heavier than warfarin-susceptible individuals (Smith et al., 1993).

Scents may play a role in reducing rat neophobia. If rats create a background of scent marks in their environment as mice do (section 1.3.2.1), they might be able to detect any new food or object simply because it does not smell of rat.

Social transmission of food preferences. A further interesting feature of rat feeding behaviour is that a rat can learn to select diets that another rat has eaten (reviewed by Galef, 1990a). Rats allowed contact with either the mouth, the breath of a tube-fed conspecific, or food powder on the fur of a conspecific that had eaten a new diet, increase their consumption of that food compared to another novel food (Galef and Stein, 1985). The flavour cues in

mother's milk, or the presence of a demonstrator at the site of subsequent food presentation (Galef, 1990a) also induces diet selection. This effect can also be produced by a combination of scents (urine, faeces and foot gland secretions) left by rats previously feeding at a food bowl, though none of these scent sources when presented alone results in social learning of the diet (Laland and Plotkin, 1991). Environmental cues, such as a cotton swab powdered with food, can induce diet preferences (Posadas-Andrews and Roper, 1983), although these results seem to be contradicted by later replications (Galef, 1990b). Social learning of diets appears to be mediated by odours, since anosmic rats cannot learn socially-mediated food preferences (Galef, 1988).

Diet selection through social learning is stronger among juveniles than among adults (Galef, 1977). Juveniles are unable to gain weight when having to learn to select rich food diets for themselves (Beck and Galef, 1989), but can do it as a consequence of social learning when an adult rat, a 'demonstrator', is present. Adults can also gain from learning from other conspecifics: rats in groups gain weight faster than isolates when having to select a protein-rich diet from among another 3 poor diets (Galef and Wright, 1995).

Food aversion has also been found to be socially learnt (Galef, 1986a). Although naive rats interacting with a poisoned conspecific may not learn to avoid the demonstrator's diet (Galef, Wigmore and Kennett, 1983), the evidence shows that rats can learn social aversion. When laboratory rats that have been fed on two novel foods are made ill through an injection with lithium chloride (LiCl) and then are presented with a demonstrator, observer rats only develop avoidance to the new food that has not been eaten by the

demonstrator (Galef, 1986a). Avoidance of a new food which has produced toxicosis is reduced when a demonstrator eats the new food in front of rats that have been made ill (Galef, 1986b).

Rats can store and use information about diets for 12 hours at least (Galef, 1983). They can detect 3 to 4 different flavourants eaten by demonstrators (Galef and Whiskin, 1992). Social learning may result in the cultural spread of food preferences within a population, since chains of at least 8 transmission episodes can be established by allowing an observer rat to be a demonstrator for the next rat (Laland and Plotkin, 1990, 1992).

There is one documented case in which cultural transmission of diet preference and food handling in a wild population may have occurred (Galef, 1980). Rats in a locality in Italy are known to dive for molluscs. However, Galef (1980) could not induce social learning of diving for food in the laboratory.

1.4.7. Communication.

Although some examples have been discussed on the role that scents play in communication, most of what has been published about the rat communication system (particularly olfactory communication) will be discussed in the following sections.

1.4.7.1. The use of ultrasounds in communication.

Rats produce ultrasounds in two bands: 22 kHz and 50 kHz. Both seem to play a role in sexual communication and in aggressive contexts (Barfield et al., 1979; Corringan and Flannelly, 1979).

Ultrasonic calls are used in sexual communication (Geyer, Barfield and McIntosh, 1978; reviewed by Barfield et al., 1979). The 22 kHz call occurs before and after ejaculation and in other phases

of mating (Adler and Anisko, 1979). Twenty two kHz calls seem to indicate that the male is in the refractory period after ejaculation, whereas 50 kHz calls are emitted when the male solicits the female or when he mounts her (Barfield et al., 1979).

Males call, shifting from the more common 50 kHz call to the 22 kHz one, in the presence of receptive females: the more receptive a female is, the longer the time the male spends calling and the greater the rate of the call (Geyer and Barfield, 1978). The production of ultrasound appears to induce or increase female darting behaviour (an approach-flee response preceding mating). Fifty kHz ultrasonic calls broadcast through a speaker increase female darting behaviour in the presence of a castrated male (McIntosh, Barfield and Geyer, 1978). They may also play a role in mate choice as, in a two-choice test, darting behaviour is directed towards a castrated male with a speaker broadcasting ultrasounds in preference to a castrated male without a speaker (Thomas, Howard and Barfield, 1982). Male rats also emit the 22 kHz vocalisation after mating to exhaustion; this appears to keep the female away from the male without causing her to leave (Barfield and Geyer, 1975). In this context, calling may signal social depression and a withdrawn state (Anisko et al., 1978).

Twenty two kHz calls are also produced in highly stressful situations such as the experience of an electric shock or agonistic encounters (Adler and Anisko, 1979). In the latter case, male rats produce ultrasounds in the presence of other male rats which have repeatedly defeated them (Corrigan and Flannelly, 1979). In such situations, calling may signal submission. Aggression is reduced in encounters where long ultrasonic pulses are artificially broadcast (Sales, 1972). Naive residents also decrease attacks (increasing the

latency to the first attack and reducing the duration of the offensive behaviour) towards calling intruders (Lore, Flannelly and Farina, 1976).

Rats emit 22 kHz ultrasonic vocalisations in the presence of a cat, but only in colonies where burrows are available. Individually isolated rats do not show this behaviour (Blanchard et al., 1991). Ultrasonic calls thus may be used as an alarm signal, indicating the presence of danger. However, as rats living in colonies may sustain aggression especially when they have burrows, whereas isolated rats do not (Blanchard, Blanchard and Flannelly, 1985), the cause of the observed difference in calling may be aggression from conspecifics in the colony situation.

1.4.7.2. Scent marking and communication in rats.

Scent marking seems to play a number of roles in the rat communication system, some of which have already been discussed in the section on scent marking functions in mammals. The following sections will discuss the roles that urine and other rat scents play in orientation, individual discrimination, dominance advertising, sexual communication, attraction of offspring and alarm of conspecifics.

1.4.7.2.1. Orientation.

If urine or any other kind of scent is used in orientation, a non-uniform pattern of deposition is likely to arise (but see section 1.3.2.1.). In addition, if scents assist rats in orientation they should increase both investigation and marking in response to changes in the background of scent marks.

Rats deposit scent marks at special sites. Both laboratory rats in cages (Brown, 1985c; Hopp and Timberlake, 1983; Peden and Timberlake, 1990) and wild free-ranging rats (Telle, 1966) readily mark protruding objects encountered in their home range. In addition, laboratory rats in test cages are known to urine mark the entrance of their burrows (Hopp and Timberlake, 1983; Peden and Timberlake, 1990), whereas wild rats rub their flanks against the entrance of their burrows, stones and other objects (Calhoun, 1962), a behaviour also considered as scent marking. These may constitute examples of scent marking, but there is little evidence on the behavioural responses they elicit.

A number of observations suggest that rats may use scents in orientation: both wild and laboratory rats use pathways to move through their home range (Calhoun, 1962; Telle, 1966; Barnett, 1975; Boice, 1977; Recht, 1982). Rats mark pathways with urine in outdoor enclosures or when free-ranging (wild rats: Calhoun, 1962; Telle, 1966; laboratory rats: Boice, 1977). Similarly, rats in laboratory cages move more frequently around the periphery, avoiding the centre of the cage (Peden and Timberlake, 1990), and urine marks are much more common around these paths (Richards and Stevens, 1974; Peden and Timberlake, 1990). These urine marks may signal a safe, obstacle-free path for four reasons: i) rats usually move within or close to cover (Taylor, 1978); ii) they confine their movements to trails, and tend to divert pathways to nearby walls or vertical sheltering objects (Calhoun, 1962), leaving urine trails along safe paths which offer them some cover; iii) rats move at high speed along paths (covering, for example, up to 96 metres in 10 seconds; Recht, 1982), as if not expecting any obstacle to be in their way, but move slowly and explore while outside

pathways (Telle, 1966); iv) when rats are introduced into a vacant territory previously occupied and marked by other rats, these newcomers immediately start using urine trails as pathways, and they urine mark the trails (Telle, 1966). This suggests that urine trails provide intruders with information about how to move around the vacant territory. Nevertheless, they might follow the urine trails because these constitute an interesting stimulus. The finding that rats travel faster along their own urine trails could also be explained in terms of familiarity with the pathways, although scent marking may play some role in producing such familiarity. Additional evidence regarding the use of trails of unspecified source in orientation by laboratory rats has been found recently by Galef and Buckley (1996). Recently fed rats deposit trails that attract conspecifics. The trails are not attractive if they have been left by a rat that has not eaten. Follower individuals cannot detect the direction of the trail, but the trail is more attractive the more rats have passed along the trail.

Urine marks by the walls of a laboratory cage may also be included within the tendency of rats to use pathways near vertical objects, but it should be noted that, when rats move around an unfamiliar platform *without* walls, they also prefer to stay in corners and along boundaries even in the absence of vertical objects (Eilam and Golani, 1989).

The response of rats to manipulations of the background urine marks covering their home range is not well documented in the literature. Information regarding responses towards clean areas or manipulations of the olfactory background would provide evidence on the use of urine marks in orientation. Tests comparing marking, or investigation of clean objects compared to those

covered with conspecific urine marks have usually been conducted in clean cages. Under these circumstances, a clean object does not contrast with the substratum, and thus, it is not possible to use information from these experiments to assess whether rats respond to manipulations of the urine background.

Faeces have not been described as playing a role in scent marking the home range among rats. Boice (1977) found faeces near burrow entries. Calhoun (1962) observed that defecation occurs where locomotion is halted. He also found some clustering of faeces at the intersection of pathways and spread along trails. There is only one case in which faeces have been found to be used for communication: the so-called maternal pheromone, which will be discussed later in this chapter.

1.4.7.2.2. Individual differences in scent marking.

The literature is not consistent about differences in urine marking between male and female rats. Some researchers have found no difference (Birke and Sadler, 1984; Peden and Timberlake, 1990), others have indicated that males investigate conspecific scents and urine mark them more than females do (Brown, 1991; marking alone: Price, 1977; investigation alone: Thor, Wainwright and Holloway, 1981), while a third group found that females investigate and urine mark conspecific scents more than males do (Lee, Mitchell and Adams, 1984). Sexual differences have also been found in flank marking, where males flank mark more than females (Peden and Timberlake, 1990), and in deposition of faeces, where females were found to ambulate and defecate more than males (Gray and Lalljee, 1974). Viveros, Hernández and Gallego (1990), however, found that .males defecated more than females in stressful situations. In

contrast, it is consistently found that female rats increase urine marking at oestrus or pro-oestrus (Calhoun, 1962; Birke, 1978; Birke and Sadler, 1984; Lee, Mitchell and Adams, 1984; Matochik, White and Barfield, 1992; but see Peden and Timberlake, 1990).

Although sex differences in scent marking suggest scents might be used in communication, these differences do not conclusively demonstrate it (section 1.3.2.2.2.1). For example, greater marking could be a by-product of one sex having a higher metabolic rate than the other, resulting in greater production of scent (see discussion of mice below).

No comprehensive study appears to have related individual differences in scent production by rats to an individual's communication requirements. For example, dominant males may need to produce large quantities of urine to mark their territory. Similarly females may need larger quantities of urine to signal their receptivity when in oestrous or to warn off intruders when pregnant. In mice, however, such differences in urine production seem to be consistent with urine marking necessities of each individual and sex (Drickamer, 1995), and it is worth while mentioning it here. As some authors have found in rats (see above), Drickamer (1995) found that male mice produce urine at a higher rate than females (1.5-2.0 times greater). He found a correlation between urine production and body weight of mice. If such a correlation held in rats, male rats would have a greater rate of urine production than females, and adult rats would produce more urine than immature individuals. As in rats, female mice produce more urine at oestrus (Drickamer, 1995). This is consistent with a female's necessity to advertise her receptivity. Female mice increased urine production in the last two thirds of pregnancy and during lactation. In rats, this

period is associated with increased aggressiveness and territorial exclusion (see section 1.4.5.1) although, unfortunately, it is not known whether urine marking in pregnant/lactating rats increases as in mice. If so, pregnant/lactating rats may produce more urine to advertise their increased intolerance of conspecifics.

Drickamer (1995) did not find that urine production from individual mice sampled from a crowded group differed from that of mice living in a less crowded group or in isolation. However, subtle differences in individual mice of the same social rank living in different population densities might have been missed because individuals were sampled at random, and variability in mouse urine production was high. Finally, Drickamer found that dominant male mice produced more urine than subordinates, which is consistent with the finding that dominant male mice mark to advertise status and occupancy (Hurst, 1990a), whereas subordinates mark to gain tolerance (Hurst, Fang and Barnard, 1993). Dominance advertising should be an honest signal and therefore, it is likely to be a costly one. An expensive high marking rate may be a means of precluding subordinates from advertising dominance. However, Drickamer (1995) found that dominant female mice did not produce more urine than subordinate females. As dominant female mice compete for breeding opportunities (Hurst, 1990b), they will normally be either pregnant or lactating, producing, thus, more urine than non-pregnant females. Thus, pregnancy/lactation would provide dominant females with enough urine to signal their dominance.

The amount of secretion produced is only one way in which physiological differences could suggest communication needs. Another is the composition of the scents. Thus, in the example discussed earlier, dominant and subordinate females may produce

the same quantities of urine but dominant females may satisfy their communication needs by increasing the concentration of semiochemicals. There is very little research on this subject in rats. Finlayson and Baumann (1957) found that in both rats and mice there is a greater concentration of proteins in the urine of males than in that of females. Subsequently, Robertson, Beynon and Evershed (1993) have found that these proteins in mice bind odorants involved in communication, although nothing appears to be known in rats.

1.4.7.2.3. Discrimination of scents from different individuals.

Further evidence that a scent is used in communication derives from discrimination of scents belonging to different individuals. Female rats can discriminate odours from two different males (Krames, 1970). Similarly, male rats habituated to the whole body odour of a juvenile, increase investigation only when presented with a different juvenile not a re-exposure to the first juvenile (Thor et al., 1988). Mother rats can discriminate the sex of their pups using urine (Moore and Samonte, 1986). One origin of these differences between individuals seems to be, at least in rats, the Major Histocompatibility Complex (MHC). Male laboratory rats from the strain PVG-RT1^{u} can distinguish between urine from PVG and PVG.R1 rats, which differ only in the A region of the MHC; this is one of three regions forming the MHC (Brown, Singh and Roser, 1987). The MHC is a part of the genome differing between individuals which codes for histocompatibility antigens, and whose derived proteins can be detected in urine using bioassays (Brown, Singh and Roser, 1987). Although rats can discriminate individual

odours irrespective of the titre of sex hormones in the donor (Brown, 1988), hormones may, nevertheless, play a role in individual recognition: sex hormones influence bacterial counts in female rats (Larsen, Markovetz and Galask, 1977), and male rats have most difficulty in discriminating between PVG and PVG.R1 rats raised under germ-free conditions, whereas they do discriminate when bacteria are present (McIntosh-Schellinck, Brown and Slotnik, 1991). Thus bacteria, which are under the influence of sex hormones, seem to play a role in individual recognition. Brown (1995) has proposed a mechanism by which genetic differences at the MHC, diet and commensal bacteria may interact to produce a unique individual odour: dietary factors influence the bacterial flora, as different foods provide different amino acids for the bacteria to metabolize, the bacteria produce a pool of volatile molecules and the class I antigens from the MHC filter some of these chemicals and deliver them to the urine, producing an individually distinct odour. Because each individual has a different combination of alleles at the MHC, its diet is slightly different from other individuals (under natural conditions), and as this and a number of other factors influence the composition of the bacterial flora in their guts, each individual has an odour uniquely different from other individuals.

However, these studies only show that rats, which have impressive olfactory learning capabilities (section 1.4.1), can discriminate between different scents, but not necessarily between different individuals. There is evidence suggesting that rats, like golden hamsters and mice (section 1.3.2.2.3) may discriminate between individuals and not just between different types of scent. Exposing an individual to soiled bedding or urine from a male produces habituation to the whole body odour of the mark's donor,

but not that of a different male (Sawyer, Hengehold and Perez, 1984). However, because the scent producing habituation was presumably present also on the body of the donor, this may be testing the response between a mixture of scents one of which is familiar (urine) and a set of totally unfamiliar scents from a new male. Such tests do not demonstrate that there is necessarily individual recognition (Halpin, 1986).

1.4.7.2.4. Scents deposited by rats compared to those applied by humans.

Most of the research on rat responses to conspecific scent marks in the literature involves urine stimuli deposited by the experimenter (e.g. Brown, 1975, 1977, 1985c, 1986c, 1991, 1992; Birke 1978; Price, 1977). Authors do not seem to have considered the possibility that responses to a scent may vary depending on whether it was deposited by a rat or by the researcher. This, however, could be of primary importance because rats may change the composition of the scent according to the context in which it was laid (aggressive, sexual, etc.), or part of the information a signal carries may be encoded in the pattern of deposition (Albert, 1992). Although no author seems to have considered this possibility in the case of rats, one report found a difference between the rat's response towards stimuli deposited by conspecifics and similar stimuli applied by humans. Birke and Sadler (1984), in a series of experiments investigating sex differences in urine marking and investigation towards conspecific scent compared to blank (clean) controls, found significant responses only towards objects urine-marked by rats, and not towards those where urine was applied by the researcher. However, the authors did not discuss the importance of this finding

and the consequences it may have in olfactory communication studies.

1.4.7.2.5. Scent marking in competitive behaviour.

Olfaction plays a role in rat aggression. Anosmia greatly decreases residents' aggression towards intruders (Alberts and Galef, 1973; Flannelly and Thor, 1976a; Price, 1978; Flannelly and Blanchard, 1982). Furthermore, aggression and olfactory investigation are linked in competitive situations. Residents investigate and attack intruders more than intruders attack and investigate residents (Brown, 1992). Furthermore, males from stable hierarchies prefer the odour from cylinders that have housed strange subordinates over those that have housed strange dominant males (Krames, Carr and Bergman, 1969). Individuals show this response probably because, in a similar natural situation, being near a subordinate's nest involves less risk of aggression than being near a dominant's nest.

The best studied scent in the rat is its urine. Urine marking seems to play a role in competitive behaviour. For example, urine marking may elicit aggression and, conversely, aggression may influence the rate of urine marking. When an intruder is introduced into the cage of a laboratory rat, the resident investigates the intruder and then its own home cage (Adams, 1976). As the intruder urine marks the cage, the resident counter-marks and attacks the intruder. The intruder marks less after defeat (Adams, 1976). Evidence of the importance of the pattern of deposition and the role of marks in aggression can be found in Gawienowski, DeNicola and Stacewicz-Sapuntzakis (1976). Although individuals usually investigate male scents for longer than clean substratums

(Brown, 1975, 1985c, 1986c, 1991; Birke and Sadler, 1984), Gawienowski, DeNicola and Stacewicz-Sapuntzakis (1976) found that male laboratory rats avoid an area sprinkled with adult male urine, preferring to stay in the clean half of the cage. However, they showed neither preference nor avoidance of urine from castrate or juvenile males, or of that taken directly from the bladder of an intact male. This might be due to the effect of using urine from a mature male applied in small spots in contrast to the single spot used in other studies. Thus, an aggressive message for intruders may be conveyed by the combination of both the pattern and the quality of the marks. Consistent with this, aggressive males mark both their environment and conspecifics more than less aggressive males do (Taylor, Bartko and Farr, 1987).

Male intruders are attacked more often than females by dominant males (Barnett, 1958; Calhoun, 1962; Alberts and Galef, 1973; Thor and Flannelly, 1976b; Barnett, Dickson and Hocking, 1979), probably because they compete for all resources including mates, whereas females may compete for some resources but not for mates. Thus, individuals of the same sex are expected to compete more than individuals of the opposite sex. In accordance with this prediction, aggression is mainly directed towards intruders of their own sex (Brain et al., 1980; Blanchard, D.C. et al., 1984). Accordingly, when scents play a role in communication between competitors, individuals should investigate and counter-mark scents from individuals of their own sex more than those from individuals of the opposite sex. On the other hand, when scents play a role in sexual communication individuals should investigate and counter-mark scents from the opposite sex more than those from their own sex.

Most published research on rats reports greater investigation and marking towards marks from members of the opposite sex, which suggests scents play a role in sexual behaviour rather than, or in addition to, advertising aggression. Birke and Sadler (1984) found that dioestrous females mark male urine more than female urine. Brown found that males investigate (Brown, 1977, 1985c, 1986c, 1991) and mark (Brown, 1992) female urine more than male urine, while Flannelly and Blanchard (1982) found that males also investigate female conspecifics more than males. Brown (1977, 1991) found that females investigate male urine marks more than female marks, although he did not find any preference in urine marking. Gao (1991) observed that rats were indifferent to urine of their own sex, but preferred the urine of the opposite sex to a clean control.

This research might be biased due to a number of factors:

i) The subjects were either isolated (Brown 1977, 1985c, 1986c 1991, 1992; Flannelly and Blanchard, 1982; Gao, 1991) or housed in single sex groups (Birke and Sadler, 1984; Brown, 1985c, 1991). The presentation of a female or female odours may be highly attractive for males which had no contact with females. The response of individuals in mixed sex colonies might be rather different. Aggression is increased in mixed sex colonies (Barnett, 1958) and a role for scents in aggression is more likely to be detected in this type of colony. In addition, males living permanently with females not only show sexual responses, but they may also attack the females they live with (Barnett, 1958; Calhoun, 1962; Barnett et al., 1979).

ii) Animals were kept in laboratory cages, where dominance hierarchies are less strong than among animals kept in larger enclosures (Adams, 1985; Adams and Boice, 1989). Again, when

the competitive drive is weaker females are more likely to be considered as a mate than as a competitor.

iii) Tests were usually carried out in clean cages (Brown 1977, 1985c, 1986c, 1991, 1992; Birke and Sadler, 1984) or in testing environments (Gao, 1991) which might not be seen as a home residence by the rats tested and thus would not be defended as such against intruders. Only Flannelly and Blanchard (1982) tested individuals for their response towards conspecifics in their home cage.

iv) Laboratory strains have always been used which, as discussed earlier in this chapter, are less aggressive than wild rats.

1.4.7.2.6. Scent marking in sexual behaviour.

Urine marking seems to be used in rat sexual communication. There are a number of roles that urine may play in sexual communication (section 1.3.2.2.2.2):

Mate attraction. At least two predictions arise from this hypothesis (section 1.3.2.2.2.2.1): i) females should mark more when they are receptive and; ii) males should respond to scents from receptive females more than to those from non-receptive females.

In agreement with the first prediction female rats increase scent marking at pro-oestrus (Calhoun, 1962; Birke, 1978; Birke and Sadler, 1984; Lee, Mitchell and Adams, 1984; Matochik, White and Barfield, 1992; but see Peden and Timberlake, 1990). Calhoun (1962) observed that during the night before oestrus, female wild rats wandered more than usual beyond their home range. Periodically, they stopped to rub their sides and ano-genital area on the sides of burrows, trees, stones, etc., a behaviour considered as scent marking.

In agreement with the second prediction, Calhoun (1962) reported that male wild rats were attracted to these marks, which they keenly investigated and counter-marked, and followed the female seeking an opportunity to mate. Male laboratory rats tested in clean arenas increase marking and investigation in response to urine from oestrous females compared to urine from other females (Carr, Wylie and Loeb, 1970; Lydell and Doty, 1972; Lee, Mitchell and Adams, 1984; Merkx, Slob and Van der Werff ten Bosch, 1988; marking alone: Hopp and Timberlake, 1983; Birke and Sadler, 1984; but see Gao, 1991 and Natynczuck, 1990; investigation alone: Stern, 1970). The attractive factor in oestrous females is found in preputial gland extract (Gawienowski, 1976; Thody and Dijkstra, 1978; but see Merkx, Slob and Van der Werff ten Bosch, 1988) and has been suggested to be an aliphatic acetate (Stacewicz-Sapuntzakis and Gawienowski, 1977). Male urine may also be an attractant for receptive females, although it seems difficult to discern between this role and other sexual communicatory roles for male urine, such as mate choice and assessment.

Reproductive synchronisation of mates. Urine or other scents may influence the reproductive physiology of females to increase fertilisation efficiency. Antz-Vaxman and Aron (1986) found that female rats exposed to bedding material scented by males before copulation increased their ovulation rate compared to control females. Olfactory bulbectomy prevented this increase. Male urine may also shorten the female oestrous cycle. Male urine sprinkled twice daily in the home cage reduces the female's oestrus cycle from 5 to 4 days (Aron, 1975).

Another finding suggests that scents are involved in reproductive synchronisation. Urine increases the stimulating

effects of male rat ultrasounds on the sexual responsiveness of receptive females (Geyer, McIntosh and Barfield, 1978). Geyer, McIntosh and Barfield (1978) also found that females urine mark and increase investigation of a male when his urine is present. Male rats of laboratory strains urinate when a female is first introduced in the testing cage, and later counter-mark female urine marks frequently (McIntosh, Davis and Barfield, 1979). Several findings suggest that this is a scent marking behaviour (section 1.3.2.2.2.1). Dewsbury (1967) found that females investigate the substratum more than males during sexual encounters. These findings suggest that male urine is used as a stimulant during copulation, perhaps to increase the ovulation rate observed by Antz-Vaxman and Aron (1986).

Scents may also produce reproductive synchronisation of individuals of the same sex as the donor. Undetermined olfactory cues produced by females result in synchronisation of oestrous cycles when 5 or more individuals are placed together or the air all of them breathe is mixed (McClintock, 1978). Female urine sprinkled twice daily on the cage of grouped females also shortens their oestrous cycle from 5 to 4 days (Aron, 1975).

Mate assessment and mate choice. Urine marking may play a role in mate choice. Male rats prefer the odours of female rats that have not copulated over those that have (Krames and Mastromatteo, 1973). As a male rat's investment in gametes has been shown not to be trivial (Dewsbury, 1982), they may obtain a greater benefit by inseminating unmated females because, in doing so, they will avoid sperm competition and will sire more offspring.

As discussed in section 1.3.2.2.2, artificial scents (perfume) not related to any genetic, physiological or resource holding potential of

individuals may influence mate choice. Under natural conditions, however, scents are more likely to give the choosing individual information about one or more of these aspects. No research appears to have been conducted on the role that scents may play in assessing parasite load in rats, as has been found in mice (see section 1.3.2.2.2). However, Brown (1995) has suggested that, because MHC antigens influence rat urine odours and their immune response, it may be involved in mate choice, signalling individuals with resistance to illnesses by means of olfactory cues.

Because females are the sex investing most in their offspring, they are usually choosier than males (Partridge and Halliday, 1984). Females should be more likely to select mates with phenotypic superiority or greatest resource holding potential. Both qualities seem likely to converge in the most dominant or aggressive male. Female laboratory rats mate more with the most dominant male in a dyad (Thor and Carr, 1979; Carr et al., 1982; McClintock, Anisko and Adler, 1982). They show the same preference when urine marking males. Females preferentially mark the most aggressive male with the highest testosterone level in a dyad (Taylor et al., 1984), which is usually the dominant individual (Blanchard et al., 1993). Finally, they also show the same preferences when presented with male urine alone. Taylor, Haller and Regan (1982) and Taylor et al. (1984) found that females investigate and mark an area vacated by a high-testosterone male more than one with low-testosterone titre, even when both males mark at a similar rate. Thus, it seems that females can assess the quality of the urine independently of its quantity. Nevertheless, dominant higher-testosterone titre individuals appear to mark more than other males. Taylor et al. (1984) found that males with high testosterone levels mark females more

than those with lower testosterone levels, and Taylor, Griffin and Rupich (1988) found that marking of the environment is correlated with marking of conspecifics.

There are, however, some reports apparently contradicting some of these results. Birke and Sadler (1983) found that females in pro-oestrus preferred an area vacated by a low rate marking male, which may imply either a mate preference for a subordinate male, or an intruder's preference for an area belonging to the less aggressive male. Berdoy, Webster and Macdonald (1995) failed to find a relationship between testosterone titres and dominance status in wild rats.

The finding that factors affecting mate choice also affect investigatory preferences also suggests that scents play a role in mate choice. Thus, female rats reared in groups investigate urine from intact males more than urine from castrated males, whereas females reared in isolation show no preference (Brown, 1991). Familiarity with a partner also seems to be a factor in mate choice and scent investigation, although its fitness consequences are not obvious. Thus, males prefer both to mate (section 1.4.5.2) and to investigate the odour of a novel female over that of a familiar female (Carr, Krames and Costanzo, 1970; Carr, Hirsch and Balazs, 1980, but see Birke and Sadler, 1984). The case is not clear for females: some authors have found that females prefer the odour of a familiar male over that of a new male (Carr et al., 1979), some found the opposite effect (Birke and Sadler, 1984, Krames, 1970), but others have found no preference at all (Carr, Krames and Costanzo, 1970). These findings may just indicate a Coolidge effect (the preference for a novel parter over the familiar one), or it may have fitness

consequences. Field tests might be very useful to discriminate between these alternatives.

Mate choice should result in greater reproductive success for the selecting sex. However, there is no evidence as yet in rats. Laboratory rats do not seem to gain a reproductive advantage in terms of litter size or number of pups delivered alive, as choosy females who mated with their preferred male did no better than females mated with their non-preferred male (Taylor and Weiss, 1987). However, in a hostile environment, access to territories rich in resources is likely to increase reproductive success, and wild free-ranging female rats mating with a preferred high status male are likely to increase their reproductive success. This might be through increased litter size (as they will have resources to support a large litter) or increased investment per pup increasing their probability of survival.

There might be a specialisation in assessment of sex and reproductive status in rat scent glands. Natynczuck (1990) found that sebaceous glands on the female haunch but not those on the shoulders undergo cyclic variations during the oestrous cycle. Male rats may sample both to detect oestrus.

As discussed, scents appear to play several roles in rat sexual communication. However, anosmia does not seem to impair sexual behaviour in laboratory rats. Anosmia reduces female ano-genital investigation by males, but it increases mounting by a factor of 2 (Thor and Flannelly, 1977). However, this is not to say that anosmia does not have a detrimental effect on sexual behaviour. Anosmia may affect male and female mating through several mechanisms: i) mate choice would be impaired, if scent marks provide information for mate choice as suggested by the literature;

ii) males would not be able to detect oestrous females. Males may allocate their limited semen resources suboptimally (Dewsbury, 1982) by not ejaculating at the time when the probability of impregnating the female is greatest.

1.4.7.2.7. Factors influencing urine marking and investigation.

Several factors may influence marking and investigation which need to be taken into consideration in the design and analysis of experiments. Some of them, such as the effect of using laboratory strains in small cages have already been discussed. Others include: familiarity with the scent donor, housing and rearing regime, sexual experience and age of the scent mark.

-Familiarity with the scent donor. Males appear to prefer familiar to unfamiliar urine: an area of a test cage with urine to which male rats have become familiar attracts more males than another area with urine from unfamiliar males (Fass, Gutermann and Stevens, 1978). However, in similar tests, Carr et al. (1976) found no such preference. This preference for familiar urine is reversed when investigation is aimed at females: males investigated cards scented by an unfamiliar female for longer than those scented by a familiar female (Carr, Krames and Costanzo, 1970; Carr, Hirsch and Balazs, 1980). Female rats also seem to prefer marking (Birke and Sadler, 1984) and investigating (Krames, 1970) an area marked by an unfamiliar male more than one marked by a familiar male, although females show no preference if tested after mating (Carr, Krames and Costanzo, 1970; Carr et al., 1979). Familiarity is likely to have special importance in studies involving competitive or territorial responses. Fisher (1954, see review by Temeles, 1994) has

suggested that the threat from unfamiliar intruders, which are not likely to have an established territory, is greater than the threat from neighbours, which are already established. The main threat from neighbours is likely to be that they may mate with resident females. Thus, aggression against unfamiliar intruders should be greater than towards neighbours, and this is likely to be reflected in the rat's response to scent marks from non-residents.

-**Housing and rearing regime.** Isolated individuals may not show any investigation or marking preference (Brown, 1985c), or differ in their response compared to individuals housed in groups: isolated males investigate female urine for longer than male urine whereas males housed in groups show opposite preferences (Brown, 1991). Brown (1991) also found that isolates mark less than individuals housed in groups.

-**Sexual experience.** Individuals without sexual experience, either kept in isolation or in single sex groups, may fail to show a preference for the scents of some conspecifics over others. Thus, male rats without sexual experience do not investigate or urine mark odours from receptive females in preference over non-receptive females (Carr, Loeb and Dissinger, 1965; Stern, 1970; Brown, 1991).

-**Age of urine.** Males investigate fresh urine from oestrous females for longer than that aged 1 to 3 days (Lydell and Doty, 1972). However, Price (1977) found that they investigate male urine aged 7 to 8 days more than fresh urine. None of these authors explained why rat urine attracts conspecifics for the period of time it does. Ferkin et al. (1995) studied fade out times of two scents in meadow voles that attract members of the opposite sex, and suggested a functional explanation. They found that male anogenital scent attracts females for 25 days, whereas that of the

female attracts males for only 10 days. In contrast, male scent from the posterolateral region is attractive for only 24h. Ferkin et al. (1995) suggest that animals may use changing odour qualities of scents to estimate how recently conspecifics were in an area. In addition, they suggest that two scents that attract individuals of the opposite sex but differ in fade out times may convey different messages: long lasting scents (like those from the anogenital area in meadow voles) may serve for gender identification and for advertisement of the individual's presence, while short lasting scents (like those from the posterolateral region in meadow voles or urine from oestrous females) may help to assess reproductive condition.

1.4.7.2.8. Hormonal control of urine marking and investigation.

Sex hormones greatly influence scent marking and investigation in rats.

Urine marking of conspecifics and the environment decreases with castration in male laboratory rats and increases with testosterone restoration (Price, 1975; Brown, 1978; Taylor, Bartko and Farr, 1987; Matochik and Barfield, 1991). Some researchers have found that male and female castrates urine mark less than intact individuals or do not urine mark at all (Brown, 1977; Price, 1977; Taylor, Haller and Regan 1982). Investigation of conspecific urine or clean stimuli is also reduced in castrates (Brown, 1977; Matochik and Barfield, 1991). Although hormonal restoration treatment increases urine marking in castrated females, there is no agreement on whether this provides a complete restoration (Birke, 1984, using oestradiol and progesterone) or not (Brown, 1978, using

testosterone and oestradiol; Matochik, Barfield, and Nyby, 1992, oestradiol and progesterone).

Sex hormones may have an additional effect on olfactory communication. Gonadal hormones have been reported to influence threshold detection of scents in humans (Doty, 1986). However, no research on rats seems to have been carried out.

Unfortunately, research on hormonal control of scent marking cannot help to discern whether marking behaviour plays a role in sexual or competition advertisement because both aggression and mating are under the control of sex hormones (see sections 1.4.5.1, 1.4.5.2 and 1.4.7.2.6).

1.4.7.2.9. Olfactory cues which produce alarm or signal reward in rats.

As discussed in section 1.3.2.2.4, scents may signal alarm or threat. Several experiments (see below) have shown that rats can discriminate between stressed and unstressed conspecifics. However, the evidence for a rat scent signalling alarm seems to be weak.

Rats are sensitive to odours released by injuried individuals. They show immediate freezing and other fright reactions in the presence of blood (Hornbuckle and Beall, 1974), or blood and muscle (Stevens and Saplikoski, 1973) from conspecifics, but not from humans or other rat tissue. They might use such cues to avoid traps or other places where conspecifics died or have been killed by predators. However, these are incidental odours: it seems unlikely that any chemical in the blood or muscle has evolved for communication purposes.

Rats can discriminate between odours from stressed and unstressed conspecifics (Valenta and Rigby, 1968). Rats stop where

stressed conspecifics have run in an alley maze more often than where unstressed conspecifics have run (Stevens and Köster, 1972). This effect is induced by urine, but not by faeces (MacKay-Sim and Laing, 1981a). A similar effect has been found when the donors were not stressed or unstressed, but were expecting to find a large versus a small reward or no reward at all (Ludvigson, Mathis and Choquette, 1985; Batsell et al., 1990). Ludvigson, Mathis and Choquette (1985) and Batsell et al. (1990) found that naive rats run faster if previous rats were expecting a large reward than if donors expected a small or no reward.

However, these findings do not imply that there is a scent signalling either stress or a reward. Stressed rats urinate more than non-stressed rats (Harkins, Becker and Wright, 1974; Viveros, Hernandez and Gallego, 1990; personal observation). Thus individuals may use the higher amount of volatiles in the air when donors were stressed to discriminate between these conditions, or they may stop more often to sniff when running in an alley maze with more urine marks. A similar result may be obtained by rats expecting large rewards because donors will run faster, stopping less often to urine mark, or they may leave more scents as a consequence of staying longer in the maze.

Some studies have increased this controversy. MacKay-Sim and Laing (1981b) found that rats do not discriminate between stressed and non-stressed conspecifics by means of their body odours or blood. However, the result might be due to poor experimental design. Rats at the start of the Y-maze could sample air flowing from both arms. Presumably because they could obtain a maximum of information from this point, rats often stopped at the intersection and the trial was recorded as no selection shown by the rat.

1.4.7.2.10. The role of the preputial gland in urine marking.

The preputial gland is a sebaceous-derived scent gland with ducts opening on to the surface of the penis or the clitoris (Brown and Williams, 1972), not as has been reported into the urethra (Noble and Collip, 1941). This gland releases mainly lipids, but also carbohydrates, some proteins and amino acids into the urine (reviewed by Brown and Williams, 1972). Male rats prefer an extract from female preputial glands to that from either muscle or fat (Gawienowski et al., 1976). Females, as well as males, prefer female preputial gland compared to submaxillary-sublingual glands or foot pads (Orsulak and Gawienowski, 1972).

The preputial gland may be the source of a possible aversive factor in male urine. Rats spend less time in part of a clean cage sprinkled with urine from a mature male than in a clean area (Gawienowski, DeNicola and Stacewicz-Sapuntzakis, 1976). Bladder urine did not produce an aversive effect. This suggests that the preputial glands add chemicals to the urine which produce this aversive effect. The aliphatic acetates found in preputial gland secretions seem to be one of the chemicals involved in the aversive response triggered in males (Stacewicz-Sapuntzakis, 1977). This aversion and the fact that the preputial glands of dominant individuals are larger than those of subordinates (Brown and Williams, 1972), suggest that the preputial gland may produce scents that play a role in communication with competitors.

In addition, the preputial gland seems to play a role in sexual communication. Male laboratory rats investigate preputial glands from oestrous or proestrous females for longer than those from non-

oestrous females (Gawienowski et al., 1976; Thody and Dijkstra, 1978). In addition, Thody and Dijkstra (1978) found that males prefer intact to preputialectomised females, although Merkx, Slob and Van der Werff ten Bosch (1988) found contradicting results. Laboratory males do not prefer voided over bladder urine from oestrous females, which suggests that female bladder urine is attractive to males and when females are not receptive an inhibitor might be added by the preputial gland to counteract its attractive effect (Lydell and Doty, 1972). However, if the product of the preputial glands is an inhibitor of sexual attraction it would then be difficult to explain why preputial gland extract from oestrous females is attractive to males.

Among other factors, preputial glands are influenced by sex hormones. Preputial glands become denser and larger in males at the onset of puberty (Brown and Williams, 1972), when levels of sex hormones increase. Brown and Williams (1972) also found that production of lipids reaches a maximum around puberty in both males and females. Thody and Dijkstra (1978) found that males prefer the glands of intact females over those of ovariectomised. Injection of progesterone and oestradiol completely restore the attractiveness of the preputial glands of ovariectomised females.

1.4.7.2.11. The use of faeces as scents in rat chemical communication.

Although many mammals, including rodents, use faeces in communication (see below), very little is known about rats. I shall review the two hypotheses about the role faeces play in communication: social learning of diets and incidental deposition as a result of fear. In addition, the only known case where rats use

faeces in communication, the maternal pheromone, will also be discussed.

Many mammals use faeces as scents, usually depositing them in piles called latrines (rhinos: Owen-Smith, 1971; rabbits: Bell, 1980; Veberne and Blom, 1981; Sneddon, 1991; badgers: Roper, Shepherdson and Davies: 1986; Roper et al, 1993; ferrets: Clapperton, 1989; hippopotami: Klingel, 1991; bank voles: Rozenfeld and Rasmont, 1991; genets and Egyptian mongooses: Palomares, 1993). Although in most cases faeces seem to be used in territorial marking, they might also be used in sexual communication. In female maned wolves, *Chrysocyon brachyurus*, oestrogen and progestin have been detected in faeces (Wasser, De Lemos Velloso and Rodden, 1995). The amount of these hormones in faeces changes with the oestrous cycle, which suggests that, if maned wolves could detect the hormones or a volatile dependant on them, faeces might be used for advertising receptivity.

In rats, Calhoun (1962) observed that rats deposit faeces along their pathways, particularly at intersections. Faeces appeared to accumulate wherever motion was halted. He thus thought that the pattern of faecal deposits was a by-product of the activity of rats.

In addition to incidental deposition, defecation in rats has been considered to be a fear response by a number of researchers (Harkins, Becker and Wright, 1974; Gentsch, Lischtsteiner and Feer, 1981; Gentsch et al., 1982; Viveros, Hernández and Gallego, 1990). However, no mention is made in these reports about a possible role for faeces in communication.

Laland and Plotkin (1991) suggested that faeces around food bowls may help in promoting social learning of diet preferences.

However, neither faeces, nor any of the other single cues present in that experiment were found to bias diet selection.

There is only one well-reported situation in which faeces have been shown to be involved in chemical communication among rats: the maternal pheromone. Lactating female rats produce a substance in their faeces which attracts pups during their first two weeks of life (Leon, 1974). Day 16 pups choose any lactating female in preference to a virgin rat on the basis of olfactory cues. The caecotrophe is responsible for this attraction, not their urine or normal faeces. This is a special faecal pellet produced in the distal caecum, between the small and large intestine. Although voided faeces from lactating and non-lactating females differ in attractiveness, contents from the caecum of both are attractive to pups, which implies that anal glands play no part in the production of the pheromone. There is controversy over the precise mechanism of production of the maternal pheromone and the role that bile may play in this, but the action of bacteria is necessary for the pheromone to be produced (Leon, 1974; Moltz and Leidahl, 1977; Leon and Moltz, 1978).

Pups eat faeces from their mother (Leon, 1974). Moltz and Lee (1981) have suggested that pups may benefit by a reduction in necrotising entero-colitis (a gut disease) and perhaps by increasing their ability to absorb fat. Bile is necessary for this process, and pups do not produce enough until day 30. Since the rate of myelination of nerve cells is greater between day 15 and 30, pups may also obtain the bile and long chain fatty acids necessary for myelination from the mother's faeces.

No role has been suggested in the literature for rat faeces either in sexual communication or territoriality.

1.5. Summary.

Scent marks are adapted to the physical constraints of the environment in which the marking individuals live and to types of information that they carry. Although scents are usually deposited in a particular pattern, neither the existence nor lack of such a pattern demonstrates per se the involvement of a scent in communication. However, the active restoration and maintenance of a particular pattern when it is disturbed does suggest that the scent concerned plays a role in communication or orientation.

Scent marks may play a number of roles in communication such as territorial or dominance advertisement, mate attraction, individual recognition, or mate assessment. Often, these roles are not mutually exclusive and a scent may play several roles at the same time. The context in which the experiment is conducted (e.g., testing individuals in their home pen, the social rearing of subjects, etc.) is an essential consideration when testing each particular role. In addition, several lines of evidence may be required to establish whether or not a scent plays a particular role. For example, a greater scent marking rate by a top ranking male might suggest the involvement of such scent in communication among competitors, if this male is mainly responsible for defending the territory, but does not demonstrate this. A greater response towards scents from individuals of their own sex than towards those from the opposite sex may further suggest that a scent is involved in communication among competitors. On the other hand, a greater response towards scents from individuals of the opposite sex suggests that a scent is involved in sexual communication. It is also important to note that individuals of the opposite sex are not always regarded as mates and may be regarded as competitors and treated accordingly.

Rats constitute a good species to work with because they seem to rely heavily on olfactory communication. Behavioural differences between laboratory and wild rats indicate that it is important to use the latter as subjects in experiments which aim to assess the communication system operating among free-ranging individuals.

Rats seem to use urine as their main source of scent. Apart from the case of maternal pheromones in lactating rats, there is no evidence that rats use faeces in communication. Evidence strongly suggests that rat urine plays a number of roles in sexual communication, such as mate attraction and mate synchronisation. There is also some evidence that urine plays a role in orientation and communication among competitors. However, it is unclear: i) whether urine alone produces such responses in each case or whether this involves a mixture of urine, foot gland and possibly other scents; ii) whether the roles that urine seems to play among laboratory rats tested in clean cages are the same as those operating in more naturalistic settings.

1.6. General aims of the thesis.

Although the specific aims of each particular experiments are discussed in the appropriate sections, the research project had a set of general aims:

-To assess the role that scents play in orientation by rats, particularly urine, by experimentally assessing the distribution patterns of urine and the responses of rats to manipulations in this olfactory background.

-To assess the role urine plays in the rat communication system, particularly in communication between competitors and between potential mates, by examining responses to urine from

different individuals and any sexual differences in urine marking. One of my original aims was to assess the role of urine marks in feeding behaviour, although the lack of results from my first set of experiments prevented the development of this line of research.

-Faecal marking. The potential role that faeces maight play in rat communication has hardly been mentioned in the literature, particularly among adults, and only became apparent when observations and first experiments began. The aims of this section developed as results from other sections arose. In general, these were to assess the relevance of latrines in communication between adult rats, and to assess whether rats may potentially use faeces in communication. To achieve this, I examined the distribution of faeces and how this corresponded to the use of different sites, the responses to faeces from different individuals, the odours that stimulated faecal marking, and any sex differences in these responses.

CHAPTER TWO
GENERAL METHODS.

2.1. Introduction.

As discussed in section 1.4.2, rats constitute a good species for the experimental investigation of scent marking. They have good olfactory capabilities (Slotnick, Kufera and Silberberg, 1991), it is easy to establish colonies in a relatively small space, and there is an extensive set of knowledge on almost every aspect of their biology.

After choosing the Norway rat as the species for the study, the next decision was whether to use wild or laboratory strains. Although some researchers have argued that laboratory and wild rats are not very different from each other (Boice, 1981), or that the laboratory rat's 'inferiority' to survive in the wild disappears when it is feralised (Boice, 1977), there still seem to be some unquestioned differences, discussed in section 1.4.3. This led to the use of wild rats as more representative of the species.

How experimental animals are obtained may affect test results. Wild strains of rats can be obtained through breeding wild caught rats in the laboratory. However, those raised in the laboratory will have experienced restricted space, which might affect movement and orientation responses. They will have had a constant supply of food and water usually placed in the same point and raised above the floor, impeding their ability to urine mark these, possibly affecting how rats label their resources and compete for them. They will have had no experience of predators, and sometimes little or no social experience, which is likely to affect their social behaviour. An alternative option is to obtain the animals from the wild. These animals will have experienced a completely

normal environment prior to capture, and hence their behaviour ought to be more representative of the species in the wild. However, these rats take longer to habituate to the experimental conditions and procedures and are considerably more difficult to handle (Natynczuck, 1990). Despite the latter disadvantage, the scientific importance of using animals that would give appropriate responses led to the choice of wild caught Norway rats (*Rattus norvegicus*) as subjects.

Another key aspect that could affect the behaviour of the rats is the housing regime under which rats are maintained (section 1.4.3). Although most studies use laboratory strains of rats kept in small cages, some use either laboratory or wild rats kept in large indoor or outdoor enclosures, while there are also a few studies on wild free-ranging rats.

A major advantage of keeping rats in laboratory cages is that cages allow a great exploitation of available space being small and easily moved around. Laboratory cages reported in the literature on olfactory communication and social behaviour typically measured 0.25 m^2 housing groups of up to seven rats (e.g., Brown, 1977, 1978: 35.5 x 30.5 x 71 cm, housing 7 rats; Carr, Krames and Costanzo, 1970: 25.5 x 38 x 71 cm, housing 4-5 rats; Brown, 1992: 46 x 64 x 16 cm, housing 4 rats). Cages for single individuals were smaller measuring about 0.04 m^2, an area slightly smaller than a A4 sheet (Brown, 1974, 1985, 1991; and Corrigan and Flannelly, 1979: 18 x 24 x 18 cm).

These cages, however, may create severe over-crowding conditions for rats because individuals cannot escape from conspecific attack or harassment. Under such conditions social interactions are forced, which may result in stress and reproductive

suppression (Lobb and McCain, 1978). Furthermore, Adams (1985) and Adams and Boice (1989) found that laboratory cages do not allow the establishment of dominance relationships and produce unnaturally low levels of aggressive behaviour (Boice and Adams, 1983). Both effects mitigate against using such small cages to study the natural behaviour of the species.

Another alternative was to study free-ranging rats, whose behaviour is undoubtedly representative of the species. A few studies have been conducted on free-ranging rats (Telle, 1966; Robitaille and Bovet, 1976; Recht, 1982). Although they can provide invaluable information not available from studies on captive animals, the range of information that can be extracted from this type of study is limited. In addition, this approach rarely allows formal testing of hypotheses because this requires careful control of confounding variables, which is usually very difficult to achieve in the wild. For example, it is very difficult to ensure that a range of different individuals have been tested. Moreover, wild rats are very neophobic (Barnett and Cowan, 1976) and the introduction of any experimental objects (e.g. plates or vials holding stimuli) would almost certainly provoke avoidance (captive wild rats, in contrast, can be habituated to their captive conditions, including the experimental apparatus with which they will be later tested, when introduced to their pen). Another limitation regards the identity of the individuals studied. Neither Telle, nor Robitaille and Bovet marked the individuals under observation, which restricted the information that could be obtained even further. It is also difficult to monitor the behaviour of rats in the wild, since they are hidden most of the time to avoid predation (in burrows, under the vegetation, etc.). Such studies involve very long observation periods

and the efficiency of the work, or ratio of information-gained-per-session to time-spent-in-observation, is very small. Field observations, however, provide information that laboratory work cannot provide because in the wild, the rat social system is not set by the experimenter, whereas it is in the laboratory, which may influence or restrict the range of responses by the rats.

Because of the constraints mentioned for obtaining information, field observations were rejected as an approach in the present research project. As a compromise, a large captive wild rat colony was observed for a month to assess the typical behaviours of rats, particularly in relation to scent marking. This would then form the basis for experimental tests.

Indoor and outdoor enclosures seemed to offer the best compromise between the need to control many different factors likely to influence response in the experiments and the provision of natural conditions. Outdoor enclosures reported in the literature are usually larger than indoor ones. The outdoor enclosure used in Calhoun's (1962) classical study was the largest, 924 m^2 (30.4 x 30.4 m). A quarter of that, 266 m^2, was used by Berdoy and co-workers (Berdoy M., Smith P., & Macdonald D. W. 1995; Berdoy M., Webster J. P., and Macdonald D. W., 1995). Outdoor enclosures used by other workers measured about 75 m^2 (Boice, 1977: 84 m^2, 12 x 7 m; Adams and Boice, 1983, and Boice and Adams, 1983: 72 m^2, 6 x 12 m; Adams, 1985, and Adams and Boice, 1989: 84.5 m^2, 6.5 x 13 m). Indoor enclosures, with the exception of that used by Shepherd and Inglis (1987) and also used for my study, were considerably smaller: Barnett (1958) used enclosures of 0.6 m^2 (0.6 x 1 m), and Boice and Adams (1983) and Adams (1985) kept rats in 3 m^2 enclosures (1.5 x 2 m). The 50 m^2 enclosure used for this project

provided rats with an amount of space similar to the outdoor enclosures used by Adams and Boice; aggression levels found in such enclosures are greater than those found in smaller enclosures and also result in a stable dominance hierarchy (see section 1.4.3).

Not only size, but also the internal array of the enclosure seems to affect behaviour. The presence of burrows increases rat mortality and aggression, perhaps through an intensification of dominance relationships, compared to enclosures in which rats did not have any shelter (Blanchard, R. J. et al., 1986; see section 1.4.3). For this reason, as will be discussed below, hay bales were introduced in the enclosures to stimulate burrowing.

Experimental design for recording scent marks. Most laboratory studies record urine marking using filter paper on the floor and measure the frequency of urine marking behaviours. Some researchers have measured the extent of the surface marked, or extraordinarily, the number of urine marks on the substratum. For example, Anisko, Adler and Suer (1979) used filter paper placed on the cage floor and measured urine marking as the frequency of the event of deposition. Hopp and Timberlake (1983) and Peden and Timberlake (1990), divided the paper covering the cage floor into squares using autoadhesive tape, and then measured the number of marks per square. Taylor and co-workers (Taylor, Haller and Regan, 1982; Taylor et al., 1984; Taylor, Bartko and Farr, 1987; and Taylor, Griffin and Rupich, 1988) used filter paper, but placed it under a hardware cloth floor to prevent the rats from nibbling it. They measured the extent of urine marks using a grid. A similar array has been used in laboratory studies involving other species, such as voles (Rozenfeld, Boulangé and Rasmont, 1987; Rozenfeld and Rasmont, 1991). Birke and Sadler (1983 and 1984) recorded

urine marking directly on bare floor (as the frequency of marking behaviour).

As rats seem to feel attracted to urine mark objects, a number of papers have provided objects and measured marking on these. Brown (1975, 1977, 1978, 1985c, 1986c, 1991, 1992) used a cylinder made of hardware cloth wrapped in filter paper to attract urine marking; Price (1975, 1977) used a similar device without paper; Timberlake and co-workers (Hopp and Timberlake, 1983; Peden and Timberlake, 1990) used ring magnets and solid cylinders; other authors (Adams, 1976; Birke, 1978; Birke and Sadler, 1984; Matochik, White and Barfield, 1992) used glass dishes. Adams (1976) used Petri dishes to attract the rat's urine marking. Every week, before washing the dish, it was weighed to measure the accumulation of dried urine during the previous week. This method does not appear to have been followed by any other researcher. The most common method has been to use a grid to measure the extent of urine marks (Brown, 1975, 1977, 1978, 1985c, 1986c, 1991, 1992; Price, 1975, 1977). Other methods of assessing urine marking have been to record the event of marking (Birke, 1978; Birke and Sadler, 1984; Matochik, White and Barfield, 1992), or the number of marks (Hopp and Timberlake, 1983; Peden and Timberlake, 1990).

However, such methods were impractical in my study where it was important to keep disturbance to a minimum. Wild rats are very sensitive to disturbance (Natynczuck, 1990). For that reason, and because the present study was aimed to assess the rat's natural responses in their environment, individuals were tested in their home pens. Given the size of the pens, floors could not be covered with filter paper; even if smaller areas were covered, rats would quickly chew the paper. Objects were introduced in a pilot study to

attract urine marking, but the results obtained were poor. The objects used were metal cylinders of similar size to those reported by Peden and Timberlake (1990), but the rats were reluctant to urine mark them and this approach was abandoned.

A few studies published on scent marking have been undertaken on wild rodent colonies, either free-ranging or tested in their home enclosure. This approach suggested the experimental apparatus for my experiments. Hurst (1987), working on wild free-ranging mice, used acetate sheets as marking stations that could be exchanged and enabled comparisons of marks deposited on the marked familiar portion of the sheet with those on an unmarked clean portion. When working on captive mice colonies, Hurst (1989) covered the floor entirely with clear Perspex tiles that could be exchanged.

Because covering a 50 m^2 pen with tiles would be both expensive and impractical when the experimenter needed to enter the pen, tiles were used as sampling stations for substratum marks. Thus, one tile was placed in every square metre of open floor area (except for the area occupied by the nest and feeding apparatus, which was not sampled).

It was important to consider the material that tiles were made of as this was likely to affect the response of the rats towards them. They had to be made of a material heavy enough to prevent the rats moving them around and impossible to chew. Furthermore, if tiles were going to work as a sample of substratum marking they should have a texture similar to concrete (the material the pen floor was made of) to prevent attracting the rats or provoking their avoidance. Acetate sheets or Perspex tiles were not suitable because rats might nibble or move them, while such plastics have a slippery surface

which rats might avoid. Metal tiles were an option, but not aluminium since rats can chew this. Steel is heavy enough not to be carried about by rats, but is prone to long term oxidation and corrosion by urine acids, while rats again might avoid such a cold slippery surface. Furthermore, there were serious doubts that urine marks would be visible on steel tiles unless they were painted, but paint would be quickly scratched away.

The material finally selected was quarry stone. It had a similar texture and thermal properties to the concrete of the pen floor, did not need to be painted as the brown colour was light enough to allow urine marks to be visible, and could be washed to eliminate contaminant odours (although, as it is slightly porous, in this aspect it was worse than glass, steel or plastic). Quarry tiles were too heavy for the rats to move, too hard to be chewed and, more importantly, if they were to be a representative sampling point, did not seem to differ greatly from the surrounding concrete floor. Thus, they were unlikely to stimulate special interest.

2.2. The rat shed.

As a result of the considerations discussed in section 1.4.3, the rats were housed in a large indoor enclosure. The most suitable installations were those used by The Vertebrate Pest Control Research Unit at the Central Science Laboratory (CSL; an agency of the Ministry of Agriculture, Fisheries and Food). They generously provided their rat pens and electronic equipment for this project. The CSL at Worplesdon, Guildford, had two rat enclosures of 50 square metres (10 x 5 m) each, along with associated electronic monitoring equipment. Technical advice was provided by members of the group, headed by Ian Inglis, and seconded by Pete Smith.

The rat enclosures were built in a wooden agricultural building with a concrete floor (see fig. 2.1). The shed was bird-proof and was entered through a metal sliding door. Inside the shed there were two enclosures 10 x 5 m for the rats, side by side, making a square of 10 x 10 m.

The building had roof lights (translucent ceiling) for daylight illumination. The shed was therefore not light-proof and I could not reverse the light cycle of the rats. It also had eight 60W Fireglow bulb lamps permanently illuminated (four per enclosure), which provided the only light source at night and minimised disturbance to the rats.

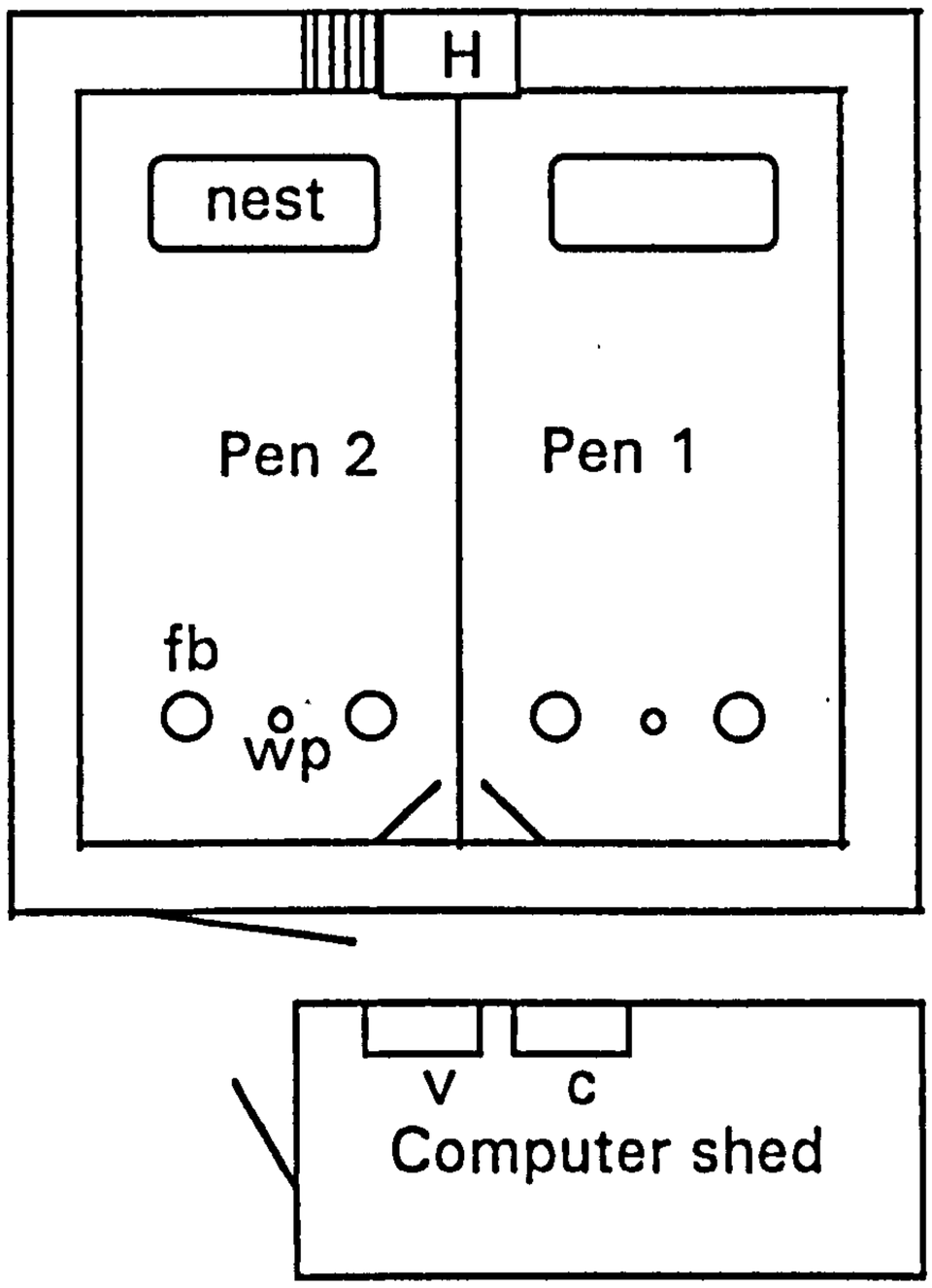


Fig. 2.1 Plan of the rat sheds. Pen 1 and Pen 2, rat pens; H, observation hide; nest, nest made with hay; fb, food bowls (large circle); wp, water pot (small circle); computer shed, shed with the video and computer equipment for the electronic balances; v, videos and monitor; c, computer connected to the electronic balances.

Windows of mesh wire along the top of two of the shed walls allowed the flow of air and some additional light to get inside the shed. A wall of Polystyrene was mounted between the top of the wall separating the two enclosures and the ceiling to reduce the air flow between the pens and thus minimising odour mixing between rat colonies.

A narrow (approximately 1 m wide) corridor surrounded the square formed by both pens.

The rat shed also had a raised wooden observation hide on the side opposite to the entrance. This was about 2 m long, 2 m high, 1 m wide (erected over the corridor, not over the rat pens) and

constructed at a height of 1.5 m. It was accessed from the corridor through a ladder and had two windows forming a protruding angle in its front wall, between both pens (see fig. 2.2). The hide enabled all areas to be monitored within the enclosures except the corners of each pen immediately below the windows, and the area of the floor next to the wall below the hut.

Each pen had two feeding points sited 1.5 m from the front wall (opposite to the nest), and 1 m on either side of the middle axis of the pen. The feeding points consisted of a circular platform (45 cm in diameter) mounted on the weighing head of a 'Galaxy 4000' electronic balance which registered changes in weight. A food bowl was placed on each balance. There were two cameras per pen (four in total) mounted on the ceiling above the food bowls. Wiring from the cameras and the four electronic balances fed to an adjacent shed which housed the monitoring and recording equipment. The video equipment consisted of several video recorders, a four way splitter, which enabled the simultaneous recording of images from four cameras on the same videotape, and a TV monitor. Each feeding point was controlled and tared from the shed and the balance data fed to a computer.

2.3. The rat pens.

The walls of each rat pen were 1.2 m high and made of sheets of zinc coated steel (which did not allow any flow of air with the area outside of the pen). On top of the walls there were additional sheets of metal approximately 25 cm wide bent inwards at about 45°. This reduced the risk of rats escaping the enclosure. The door of the pen was located near the entrance of the rat shed (next to the computer shed). There was a set of two doors that closed a segment

of the corridor which only included the accessing doors of both pens. The corridor doors were closed before opening the door of any pen to prevent rats escaping. The floor was concrete and had a drainage channel to drain water when cleaning the enclosure (see fig. 2.2).

The rat pens were connected by a metal pipe about 25 cm long which crossed the wall between the pens at floor level and at a horizontal distance of 2 m from the wall below the observation hide. The metal pipe was covered with opaque metal lids at both ends except when, for experimental purposes, they were replaced by wire mesh lids to allow odour exchange between the pens.

There was a drainage channel 9.5 m long and about 20 cm wide in the floor of each pen. This was sited about 0.5 m from the wall furthest from the neighbouring pen. The rats used this channel frequently as a pathway in their movements around the pen, probably because it gave them some shelter and reduced the perceived risk of predation.

The pens were modified by the Central Science Laboratory during the third year of study. A partitioning wall, also made of zinc coated steel sheets, was placed in the middle of each pen dividing each in two by its longest axis. The new pens (four in total) had one electronic balance each and measured 10 x 2.5 m. Only the two central pens had a door. The lateral pens were accessed using two step ladders: one placed outside next to the pen wall and the other in front just after the wall. Both were removed after each manipulation in the pen.

The new walls were also 1.2 m high and had two sheets of zinc on top of the wall (as in the central wall in fig. 2.2), about 25

cm wide and bent inwards at an angle of about 45° to reduce the risk of rats escaping the pen.

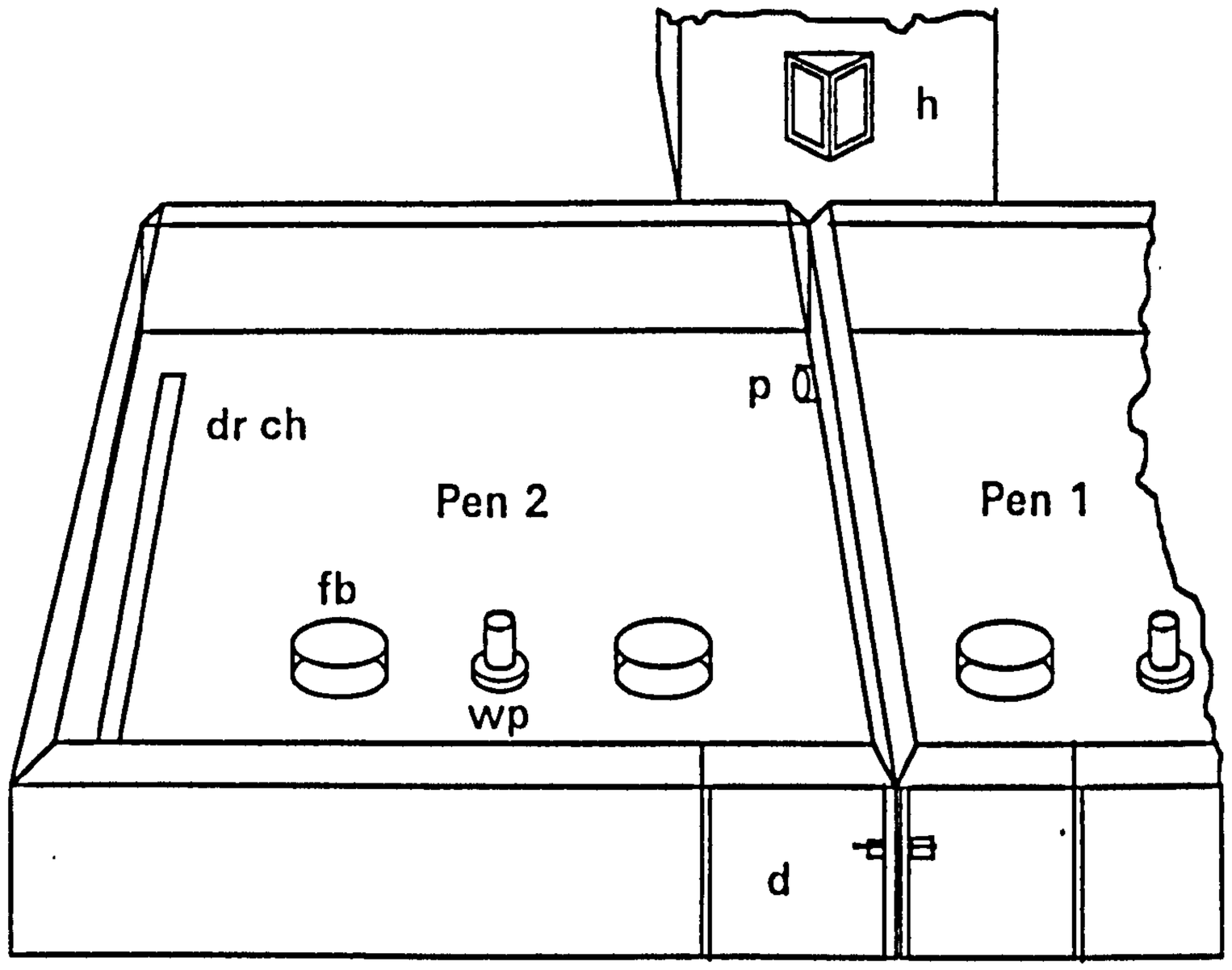


Fig. 2.2 Three dimensional view of the rat enclosures. The acronyms mean, respectively: h, hide; dr ch, drainage channel; p, pipe communicating both pens; fb food bowl; wp, water pot; d, pen door. Not a scale drawing.

2.4. Establishment of the colonies.

The rat colonies were always established from a pair of founder rats (a male and a female). Some of them were allowed to breed in order to compare data from a large colony with pens housing a pair of rats. Pairs of rats were chosen as the social unit to study scent marking experimentally for reasons discussed in section 1.4.4.2.

Each pen was provided with a stack of hay bales (1.5 x 3 x 1 m) for shelter. The hay stack was placed one metre from the wall below the observation hide. Nest boxes were placed at several

points on the hay stack. These consisted of a wood square box with a removable lid and a square hole in two of the walls (facing each other). The rats made burrows in the hay and also used the nesting boxes as chambers to sleep or hide. As the rats dug into the pile of hay, they scattered it covering nearby areas. Throughout most of the study, the hay stack occupied about 6 m^2 of the pen floor.

One food bowl (120 mm diameter x 100 mm deep) was placed on top of each balance. During the first two years, when experiments were conducted on feeding behaviour and neophobia, the food bowls were attached to an aluminium sheet 32 x 32 cm. The purpose was to allow the exchange of both the food bowl and surrounding cues (collected on the sheet) for experimental purposes. In the large colony, where pilot experiments on feeding behaviour were conducted, the feeding stations had a steel rod, 3.5 cm high by 1 cm diameter, attached to the sheet. The purpose of the rod was to present urine odours at feeding points (section 4.5). However, in most of the experiments on feeding behaviour, stimuli were applied to the edge of the bowl and not to the top of the cylinder. During the pilot experiments conducted in the large rat colony, 22 additional empty bowls were placed in the area between the balances and the front wall of each pen. These were used for a pilot experiment.

In colonies where no experiment on feeding behaviour was conducted, the food bowls were placed directly on each balance without an aluminium sheet. This set up was used during the third year, when each 50 m^2 pen was divided in two, as explained earlier, and each resulting pen had only one electronic balance. In this setting, a food bowl was placed on top of each balance and five more (also without a base sheet) were sited nearby (six empty food

bowls per pen in total). These were used for a CSL experiment which will not be discussed in this thesis.

Rats were fed with 651 Sp Rat/Mouse Breeder Cube from Grain Harvesters Ltd, Wingham, Kent ad libitum. The food came in cubes which had to be ground to powder because rats tended to hoard the cubes and, thus, reduced the time they spent outside the nest. Using powder, rats had to feed on the food bowls, which allowed more time for observation.

Each pen also had a poultry font as a water source. The poultry font was placed in the midpoint between the food balances, 1.5 m from the front wall (opposite to the hay stack). This was a water pot consisting of a cylinder with open top and with a hole in its base. The water poured from this hole into an outer ring about 5 cm high, from which the rats drank. The cylinder was covered with a cylindrical bell which had a handle on the top to transport the whole device. The bell could be attached to the cylinder with a bayonet lock. Water was supplied ad libitum.

2.5. Composition of the rat colonies.

As indicated in section 1.4.4.3, wild caught rats were used in the experiments. The rats had been caught in the wild (in farms) by the staff of the Central Science Laboratory. Hence, they had the advantage of having had a natural rearing with other rats in free-ranging colonies where they were presumably exposed to most types of social and environmental stimuli that a wild free-ranging rat has to face. The disadvantage was that no information on their prior social status and experience could be inferred. Some researchers have suggested that wild rats captured in traps are usually individuals of low social status (Boice, 1981). If so, one would expect

the rats to show reduced growth and therefore, similar light weights (as reported by Calhoun, 1962, for the lowest class, the socially displaced individuals). However, the differences in weight for rats of the same sex (males weighed from about two hundred and fifty grams to more than four hundred and fifty grams; see below) do not seem to support this idea and, according to Robitaille and Bovet (1976) such large differences in weight would result in obvious differences in the rat's social status (although, as pointed out by Berdoy, Smith and Macdonald, 1995, and Berdoy, Webster and Macdonald, 1995, smaller weight differences cannot predict social status accurately). In addition, the faecal marking response towards intruders (section 3.5 and 3.6), with some males producing a great number of faeces in all trials, also suggested that before being caught some of the males probably held a higher social status than others (section 1.3.2.2.1.1). Even if some of the individuals occupied a low social status before the experiments, their isolation in cages after their capture, and the fact that they were living in mixed-sex pairs with little or no aggression would probably have eliminated any repression induced by prior low social status.

Ten colonies of rats were used in the experiments. Eight colonies were set up by the author to test their deposition and responses to scent marks. The pattern of faeces deposited in the pen was studied in an additional two colonies utilised by the Central Science Laboratory for its research programmes. In the latter case, however, the rats had been removed when the record of the distribution of faeces was made.

Table 2.1. Weight and sex of colony members at the time of release.

colony no	status	sex	weight (g)
1	founding ind	m	550
1	founding ind	f	280
1	adult	m	450
1	juvenile	m	200
1	juvenile	m	185
1	juvenile	m	150
1	juvenile	f	175
1	juvenile	f	135
1	juvenile	f	115
1	pup	m	--
1	pup	m	--
1	pup	m	--
1	pup	f	--
1	pup	f	--
1	pup	f	--
1	pup	f	--
1	pup	f	--
2 (5 rats)	--	?	?
3 (14 rats)	--	?	?
4	founding ind	m	471
4	founding ind	f	273
5	founding ind	m	453
5	founding ind (died)	f	280
6	founding ind	m	343
6	founding ind	f	330
7	founding ind	m	370
7	founding ind	f	260
8	founding ind	m	347
8	founding ind	f	183
9	founding ind	m	313
9	founding ind	f	177
10	founding ind	m	575
10	founding ind	f	223

One of the eight colonies used in the experiments was a large colony consisting of 17 rats, the others consisted of male-female pairs. The large colony consisted of two parent founders, eight subordinates (4 males, 4 females), and a litter of seven pups (3 males and 4 females), which had not yet been weaned. The colony had been established seven months prior to the beginning of the study.

This colony was used to compare responses of rats living in a large group with that of the pairs of rats used in most of the experiments. Male and female pairs in the remaining colonies were caught from the wild and released in the pens at least one week before the start of the experimental period to allow them time to habituate to the captive conditions, and to establish their territory and olfactory mark system.

The reasons why pairs of rats were used in most of the experiments are discussed in section 1.4.4.2. Additional information on the colonies used and their period of establishment is shown in table 3.1. Weights of individuals forming each colony at the start of the experimental period are shown in Table 2.1.

2.6. General experimental set up.

2.6.1. Introduction.

All tests were conducted in the rats' home enclosure. Most of the experiments reported in the literature, in contrast, were conducted in an unfamiliar clean arena. This may have influenced greatly the results obtained (section 1.4.7.2.1): the presence of a substratum covered with familiar scent marks might affect how rats respond to clean areas, to their own marks and to those from conspecifics. The rat's own urine marks are more likely to be an interesting feature when they are encountered in a clean testing cage than when they are deposited on a background of similar marks.

Testing rats in their own rather than in an unfamiliar enclosure additionally may have deep implications for their social behaviour and thus their responses to social cues. It seems unlikely that a territorial response will be triggered in an unfamiliar cage. Scent marks may serve to form an association between the

individual and the territory it defends (Gosling, 1982). In addition to being unfamiliar with its environment, a rat placed in a clean cage does not have any scent marking cues to match with its own scents or those of a conspecific and therefore cannot identify itself as the owner or a conspecific as an intruder. In agreement with this, laboratory rats have been found to be less aggressive towards unfamiliar rats when they are tested in clean arenas than when they are tested in their home cage (Mink and Adams, 1981). Similarly, a rat cannot treat unfamiliar marks as intruder marks unless they are found in its familiar home range. Moreover, if rats are tested in unfamiliar cages, it is more likely that they will respond to individuals of the opposite sex not as competitors (as in mice, Hurst, 1990c), but as mates. Hence, a choice test between scents from unfamiliar males and females conducted in an unfamiliar arena, may indicate that rats use olfactory signals for sexual communication in this context, whereas a similar test carried out in the rat's home enclosure may indicate that those same scents are used in communication between competitors in this different situation. That is, the context changes the meaning of a signal (Inglis and Shepherd, 1990), and this needs to be carefully taken into account in both the design and interpretation of tests of response.

2.6.2. The experimental set up.

Rats were kept in individual wire mesh cages prior to the establishment of the experimental set up for two to three days. The cages, made entirely of steel mesh, measured 25 x 25 x 40 cm with a steel tray under the mesh floor. This was cleaned prior to the collection of faeces but otherwise was covered in sawdust to soak up excreta. In five of the eight colonies studied (the large colony and

four pairs), urine was collected each day from the bare tray for two days before releasing the rats in the enclosure. In four of those colonies (those consisting of pairs of rats) faeces were also collected each day. The cage tray was angled to drain the urine as soon as it was voided to prevent the contamination of faeces by urine and vice versa. Urine was stored in plastic vials (about 1 cc per vial) and faeces stored in small plastic, sealable bags (2 to 3 faeces per bag depending on the faeces' size). Samples were labelled and frozen at -20 °C until use to keep them as fresh as possible.

Laboratory rats were used as donors for urine marking tiles. The tiles served as olfactory stimuli for experiments on faecal marking reported in section 3.5. Due to an error in the supply of these rats, subadult individuals were supplied rather than adults. Since it was impossible to delay the experiment, and adult rats could not then be obtained at such short notice, I decided to use the subadult rats as donors (aged 6 weeks at the start of the experiments) despite their lack of maturity. The implications of the donor age with regards to my results will be discussed where appropriate. They were housed in RB3 stock cages with plastic base and sides and steel mesh lids (57 x 39 x 26 cm).

All wild rats to be released in the pens were marked for later identification after anaesthetising them with ether by licensed personnel. In the large colony studied during the first year, adults and subadults were fur clipped and freeze marked (Franklin and El-Absy, 1985; Hurst, 1988) with liquid nitrogen, whereas juveniles were only fur clipped to avoid the risk of skin damage from the liquid gas. As the identification of the animals became difficult at the end of the experimental period (four weeks later), subsequent pairs of rats were marked using hair bleach to discolour their fur. In

the third year of the study, the rats were marked by a scientist of CSL before my arrival. Unfortunately the hair bleach, which was applied only to males, proved too dim this time for reliable identification. Consequently, the females were fur clipped using an electric hair clipper to create patches of bare skin. After waking up from the anaesthesia, the rats were kept in individual steel cages for one day to ensure total recovery before being released into their pens. Before releasing the rats, the pens were swept out, vacuum cleaned and washed with water and liquid detergent (of the type used for dish washing). Especial care was taken to eliminate all possible faeces and urine odours left by previous rats as thoroughly as possible. The rats were left to habituate for one week after their release before starting the experiments. This time seemed enough for social interactions, as dominance in pairs of rats of the same sex is established within 10 days (Flannelly and Lore 1975).

The behaviour of the rats was recorded on video using closed circuit TV cameras. Most of them were black and white infrared-sensitive cameras for recording under dim light conditions. One of them, used for close up monitoring of urine marking over a clean tile incidentally was a colour camera, although its sensitivity equalled the former.

To improve recording visibility, an extra 60 W Fireglow red light was placed either at the sites being video recorded by the enclosure wall or at 1.5 m over the midline between both feeding bowls (in those experiments conducted in the undivided 50 m^2 enclosures). The images were recorded either on standard video recorders (during the first two years of the project), or on a time lapse video (Panasonic AG-6024, a video recorder for security systems, during the third year of the study). Images from four

cameras were fed into a four way splitter and a time printing device (except when using the time lapse video recorder, which contained its own time printing array) before sending them to the video recorder. Thus the image on the video tape was a composition of four images from different cameras, and showed the date, hour, minutes and seconds when the pictures were taken. In some cases (when monitoring close up urine marking for example) the image from only one camera was recorded in order to make measurements of urine marks on the TV screen. Most tapes were analysed on the same day they were recorded to feed the data into the computer and thus be able to re-use them. However, experiments involving food bowls were video recorded, stored and analysed in the months after the experiments due to their greater difficulty and the amount of work involved in extracting the data from each tape.

Behaviour was analysed by only one observer, as were all the rest of the procedures. During video decoding of the data almost all events were analysed more than once, to corroborate the identity of the rat involved in the response, the exact details of the behaviour or the timing of the response. Sequences of behaviour in which the identity of the individuals involved was not clear, were recorded as anonymous. They were later discarded during data analysis. Timing mistakes were examined individually and corrected if possible or else discarded. Both types of discarded data constituted only a small proportion of the analysed data.

Urine marks were collected on tiles. These were 20 x 20 cm quarry (terracotta) tiles. This type of tile was chosen for reasons discussed in section 2.1. Tiles were used as sampling sites to study urine marking of the substratum or as experimental sites to present

stimuli from rats (urine or faeces) or to assess the response of the rats to clean substratum.

The tiles used for the pilot experiments on urine marking had a steel cylinder 3.5 cm high screwed in a screw which was, in turn, glued with araldite on the centre of the tile. The cylinder was painted (white) to avoid corrosion of the metal. The aim of using these cylinders was to attract urine marking as mentioned by some authors (Peden and Timberlake, 1990). However, because the rats failed to mark most of the tiles I feared that the cylinder might frighten wild individuals and it was eliminated from subsequent designs. In pilot experiments, urine marks were recorded with a 0.25 cm^2 grid photocopied onto an acetate sheet, with a hole in the middle to allow the cylinder through. Urine marks were recorded by counting the number of squares totally or partially covered with urine. A torch was used to improve the visibility of the marks. However, the urine marks were difficult to see through the acetate and, in subsequent designs, it was discarded in favour of a different type of grid described below.

In almost all the experiments the quarry tiles were used without the cylinder. A tile was placed in the middle of each square metre of the enclosure (except those occupied by the hay stack and feeding points). At the perimeter of the enclosure, the tiles were placed adjacent to the wall to compare urine marking at the edge of the pen with that at its centre. A grid of 0.56 cm^2 (0.75 x 0.75 cm) was used to record the marks following the same procedure cited earlier. The grid was made using a 20 x 20 cm wood frame with nails at 0.75 cm intervals. Nails opposite each other were joined with fishing line inked in black. This grid allowed a far better visibility of the urine marks than the previous version.

A 0.25 m^2 grid was used to map the location of faeces in the pen and count all faeces within each square. The grid was made with a 1 m^2 frame of iron angle divided by two wires into four squares. Each wall of the enclosure was marked at 1 m intervals at the floor level, which served as a guide to place the iron angle grid.

All materials available to the rats were manipulated with clean rubber gloves. This served to standardise the odour I presented to the rats through unavoidable manipulation of bowls, tiles, etc., and avoided contamination with urine odours that might confound responses. Forceps were used to manipulate faeces at all times. Tiles were manipulated by holding them from the edges or the bottom side. After their use, they were thoroughly washed with a scrubber and detergent to eliminate urine marks, and then left to dry in the air for one day.

2.7. Human safety precautions.

Gloves also served to minimise the risk of the transmission of infections and parasites carried by rats to the experimenter. Due to the long term association between rats and humans, many micro-organisms and worms have adapted to use these rodents as a vector to infect humans. One of the most important diseases carried by rats in the developed world at present is Weil's disease, a type of severe jaundice caused by a spirochaete and carried by the urine of rats. In addition to the use of gloves, the risk of infection imposed a requirement for thorough hand washing immediately after concluding any procedure conducted within the pens. Whenever rats had to be moved from one pen to another, special protective equipment was used. This consisted of high boots and a plastic overall, with a rear air filtering device supplying air to a plastic

mask. This prevented exposure to fungi growing in the hay stack and the risk of flea bites, which was not negligible since once I discovered fleas on my white overall when cleaning the pens.

Handling procedures for wild rats. Because wild rats are disturbed very easily and are very aggressive, handling procedures had to be extremely cautious. Wild rats were never manipulated or caught with bare hands or with the sole protection of rubber gloves, unless anaesthetised. Whenever an individual had to be handled it was pushed into a black cloth bag directly from its cage. Here, the rat could not see anything and did not bite. From this bag, the rat was usually introduced into an anaesthetising chamber by licensed personnel. Wild rats were weighed, fur marked or inspected only if they were anaesthetised.

It was also risky to handle the steel cages where rats were confined. Rats squeaked, chattered their teeth and attempted to bite the researcher when handling the cage, collecting urine or filling up the food baskets. Hands were always kept at a safe distance when conducting these procedures. Cages had metal plates in the sides to prevent rats attacking humans when handling the cage, and, in addition, a steel sheet could be inserted into the cage to separate the rat from the door before opening it. To release the rats into their pens, the rat was kept away from the cage door using the partitioning sheet. Then the cage was introduced into the pen, and the door carefully opened. Finally, the partition was removed and the rat walked out.

Rats only attempted to bite, jump or attempt any other form of attack, when they were in cages or when their nest was removed from the pens to trap them (i.e., when they were at bay). No rat attempted to attack me when I observed them by sitting inside the

pens, despite the fact that they approached me. Neither did they try to attack just after being released, although they seemed very distressed a few seconds earlier. On the contrary, they moved around slowly, exploring, and some of them even turned back a few centimetres towards where I stood and observed me for a few seconds before disappearing into their nest.

CHAPTER THREE
FAECAL MARKING.

3.1. Introduction.

Faeces are one of the most commonly used sources of scent in chemical communication. It is not very difficult to see how this role in communication may have evolved. Faeces carry information about the diet the animal has eaten (Laland and Plotkin, 1991), the sex hormone levels in females (Wasser, De Lemos Velloso and Rodden, 1995), and probably physiological information concerning the health state of the donor (section 1.1.1). It is not surprising that conspecifics have evolved the ability to detect such cues through olfaction and thus, that faeces have evolved a role in chemical communication (Albone, 1984). Further, it is not surprising that faeces play many roles in communication in many mammal species because, as with urine, faeces have to be excreted anyway.

Faeces have several advantages over other possible sources of scents for carrying certain types of chemical information. Firstly, as they consist of undigested remains of food, they seem to be the best medium for providing information related to diets. Thus, Galef (1990a) and Laland and Plotkin (1992) have found that faeces add to other olfactory cues in inducing naive conspecifics to choose the diet eaten by a previous rat.

Also, as faeces have a smaller surface/volume ratio than urine and other scents that are spread thinly onto the substratum, they have a smaller diffusion rate, i.e., they release volatiles more slowly. A smaller diffusion rate implies a longer fade-out time, which, in turn, is a characteristic necessary for territorial marking for example (Alberts, 1992), because range marks should last long enough for

intruders to detect them in the absence of the signaller. That is the case in bank voles, where faeces play a role in advertising occupancy (Rozenfeld and Rasmont, 1991). To increase their efficiency as signals further, faeces are sometimes piled up in latrines. Latrines have not been reported in rats, but are formed by European badgers, which uses faeces to demarcate their territories and to advertise defence (Roper, Shepherdson and Davies, 1986; Roper et al., 1993). One of the best known examples of faecal marking is by rabbits which deposit a certain proportion of their faeces at latrines (Mykytowycz, 1968; Mykytowycz and Gambale, 1969). These faeces have an odour that smells more strongly to humans than faeces deposited elsewhere (Mykytowycz, 1968) and seem to have more anal gland secretion (Sneddon, 1991). Faeces are important in the recognition and defence of the home territory. Male wild rabbits introduced in a neutral arena together with their own faeces have a greater probability of attacking and of winning a fight than their contenders (Mykytowycz, 1973; Mykytowycz, 1976).

A sex bias in anal gland size further suggests the involvement of anal gland secretion and associated faeces in advertising territoriality. Male rabbits possess larger anal glands than females and it is primarily males which make use of anal gland secretion in marking their territories and maintaining latrines (Mykytowycz and Gambale, 1969; Mykytowycz, 1970). Male rabbits of different social rank differ in their response towards latrines. Dominant individuals investigate unfamiliar latrines introduced into their home pen from the wild more than young individuals do (Mykytowycz and Hesterman, 1970). Furthermore, faeces used in chemical communication are deposited only by the dominant male (Hesterman and Mykytowycz, 1968). Faecal counter-marking has

been suggested in rabbits: faeces deposited in response to conspecific odours are different from the rest (Hesterman and Mykytowycz, 1968); dominant rabbits also defecate over urine samples (Bell, 1980), i.e. they counter-mark urine using faeces.

There has been little research concerning faecal marking in rats (section 1.4.7.2.11). There is no evidence of faecal marking. Most researchers have considered defecation as an indication of fear (Harkins, Becker and Wright, 1974; Gentsch, Lischtsteiner and Feer, 1981; Genstch et al., 1982; Viveros, Hernández and Gallego, 1990). As cited earlier, Galef (1990a) and Laland and Plotkin (1991) found that faeces, in conjunction with other cues, play a role in the social learning of diet preferences. In only one study have faeces on their own been shown to play a role in communication: faeces from lactating females attract pups during the first two weeks of life (Leon, 1974; section 1.4.7.2.11). However, there is no evidence showing that such faeces are deposited *in order to* attract pups (thus being a signal).

No authors working with rats have suggested a role for faeces in advertising territory occupancy or defence, or advertising social status, or any form of sexual communication (mate attraction, mate assessment, etc.). As mentioned before, latrines have not been reported as such either, although some researchers have reported incidental clusters of faeces (Calhoun, 1962; Boice, 1977). Calhoun (1962) found more faeces at path intersections or wherever rats stopped. He interpreted clustering of faeces as an incidental accumulation due to the longer time that rats spent at those points, without considering that faecal clusters could be a cause of rat attraction to those sites. Boice (1977) found twice as many faeces above ground as he found inside the burrows. This would be

expected if faeces are used in warning intruders, although a number of alternative explanations are possible. In addition, Boice (1977) found some clusters of faeces near the burrow entrance, which, as mentioned earlier (section 1.3.2.1), might suggest that they play a role in orientation or social communication.

Because faecal communication is virtually a new field in rats, the research reported in this chapter started from the most basic aims: to assess whether faeces are clustered into groups which might form latrines; to assess whether any such clusters are formed deliberately by rats or are a product of incidental deposition; to assess the rat responses to faeces from different individuals and faecal marking responses to conspecific scents; also to assess any sex bias in those responses.

3.2. Pattern of faecal deposition.

3.2.1. Preliminary observations.

As discussed in section 2.1, field work on free-ranging rats tends to consist mainly of a set of observations. This approach provides interesting and useful information, but in most cases experiments are needed to discriminate between different hypotheses, and experiments are very difficult to carry out in the field (for reasons outlined in section 1.4.3). However observations may constitute a source of ideas on which to base experiments. Conducting a research project where most information has been gathered from the literature without prior observations excludes the possibility of finding distributions of marks, scent marking behaviours, etc. that nobody else has reported or considered important before. In order to obtain such insight, a large colony of rats was observed in their home pen.

The large colony consisted of 17 wild Norway rats (for details of the individuals see section 2.5), studied in its home pen before being transferred to an adjacent pen for subsequent experiments. These observations were made before transferring the rats because their behaviour was likely to be altered after the transfer by the novelty of the enclosure and experimental set up. Furthermore, I was interested in the pattern of distribution of scent marks and any behaviour related to such an established pattern. The colony had been housed in the same pen since its establishment seven months before and, since then, the pen had not been cleaned or disturbed.

A five hour observation session (2000 to 0100 h) was conducted every day for a month. At the beginning, observations were made from a hide above the enclosures. However, this perspective did not allow the front part of the hay stack (from where the rats usually came out) to be monitored (see fig. 2.2.), nor the corners below the hide or the boundaries of the wall separating both pens or the boundary of the wall below the hide. These were the most interesting areas because they were frequently visited by rats, and they showed what looked like signs of scent marking. For this reason, in most of the observation sessions I sat in a chair inside the enclosure by the wall opposite to that of the hide and near the food bowls (see fig. 2.2 and 3.1). Thus, all areas in the enclosure were monitored during the observation period. My presence did not seem to disturb the rats in any noticeable way (except if I moved), as they spent long periods of time at the feeding bowls near me, and, on some occasions, the rats even approached me. Furthermore, my presence did not seem to be the cause of the lack of faecal deposits in the feeding bowls because no such faecal deposits were observed

in this or other colonies when they were left undisturbed (see below).

The most conspicuous candidate for an olfactory cue seemed to be faeces. These were scattered throughout the enclosure at low densities (only a few per square metre). However, the density was higher along the side walls, where there also seemed to be urine trails similar to those described by Calhoun (1962), Telle (1966) and others. Faecal density was especially high in the corners where the trampling of the rats converted old faeces into a layer of faecal material over which fresh faeces were deposited (plate 3.1).

Plate 3.1. Cluster of faeces found during the observation period. They were later confirmed to be latrines built by rats.

Rats very often visited these groups of faeces first thing after emerging from the hay stack. As they came to them they slowed down to sniff at different parts of the ground (including the faeces)

or stopped at them for several minutes while engaging in self grooming. They also visited or stopped at these clusters of faeces during their trips around the enclosure.

There appeared to be no relationship between the amount of faeces deposited and the time spent in each location. Although rats visited the clusters of faeces quite often, they stayed on them for short periods (usually less than 1 minute, although occasionally they stayed for about 5 minutes). In contrast, they stopped at the feeding bowls and water pot for periods of ten minutes to half an hour, but these were almost devoid of faeces. The clusters of faeces seemed to contain a considerable proportion of the total number of faeces.

There also appeared to be signs of urine marking. Rats seemed to move along the side walls during their excursions more often than anywhere else. The white colour of the floor paint appeared darker along these paths. The most likely explanation for this discoloration was a greater rate of urine marking alongside the walls, where dust would impregnate urine and accumulate more than in areas with few marks.

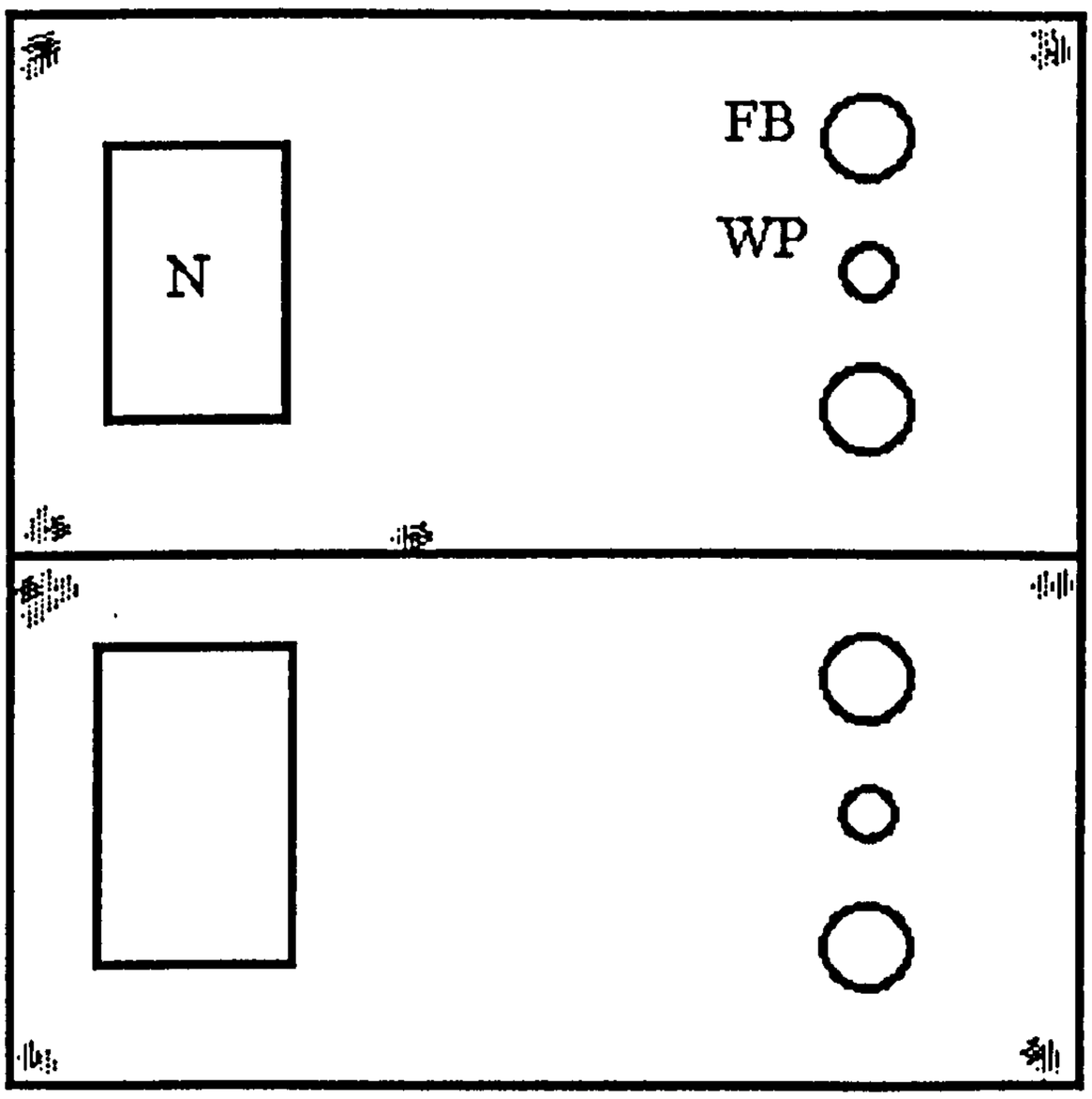


Fig. 3.1 Rat enclosures. Each enclosure was 10 x 5 m and included a hay stack for nesting (N), two electronic balances with food bowls on top (FB) and a water pot (WP). The stains at the corners indicate locations with clusters of faeces.

3.2.2. Aims.

The first step was to check the build up of the faecal deposition pattern starting from a clean enclosure. The aims were: i) to confirm that the rats built these clusters themselves, which looked like the latrines found in rabbits and other species (sections 1.4.7.2.11 and 3.1), and that the CSL personnel had not rearranged the rat faeces inadvertently into clusters as they moved around inside the pen; ii) to examine in detail the faecal deposition pattern through time for one colony of rats and to corroborate its generality by measuring the spatial distribution of faeces in several other established rat colonies.

3.2.3. Methods.

The large colony of rats was transferred to a clean enclosure neighbouring the one previously occupied. The enclosure was set up as before except that fifteen tiles (section 2.6) were placed between the feeding points and the hay stack for a set of experiments reported in section 4.6. Four additional tiles were placed along one of the side walls, and twenty food bowls were placed between the feeding points and the distal wall for experiments reported in section 4.5. The rats were seen moving around the whole enclosure without apparently being deterred by any of the objects placed inside the enclosure. Thus, to avoid disturbance of their environment the tiles and food bowls were not removed when recording the distribution of faeces.

The pattern of faeces deposited in open areas (i. e., excluding the hay stack or areas covered by hay where faeces could not be seen without causing considerable and unacceptable disturbance) was recorded every two days for 30 days. The location of faeces was

measured by counting the number found in every square of a 0.25 m^2 grid (section 2.6). Because some areas which were initially clear were later covered by hay scattered from the stack by the rats (about 10% of the open areas), the statistical analysis included only those areas which remained clear of hay throughout the mapping period.

Faecal deposition patterns were also recorded in 6 additional colonies to corroborate the pattern found in the large colony (table 3.1). In colonies 2 and 3 the pattern was recorded only once and after the resident rats had been removed. The enclosures housed 5 and 14 rats respectively for seven months. Records were taken in four enclosures housing pairs of rats. In these cases, the pattern was recorded only once after 3 (colonies 6 and 7) or 4 weeks (colonies 3 and 4).

The records were analysed by computing the dispersion index $\sigma^2/\mu \pm SE$ (Fowler and Cohen, 1990) which gives a score of 0 if distribution is regular, 1 if it is random and greater than 1 if it is contagious or clustered. This was used rather than the rate of deposition at each point because the hypothesis being examined was whether the pattern of deposition was clumped and stable. Increased deposition rates at latrines may suggest that the rats were clumping faeces more through time, whereas the dispersion index examines whether latrines held a similar proportion of faeces.

3.2.4. Results.

Clusters of faeces were found in all corners of the enclosure. They were also found by a lateral wall next to the hay stack. These clusters consisted of dense groups of faeces within areas of less than one square metre and often only a quarter of that area. Because only I entered the enclosures and I took great care not to move the

faeces, the clusters of faeces must have been formed by rats and were not created accidentally by research personnel. Thus, I called them "latrines" and will refer to them subsequently by this term.

Throughout the 30 day mapping period in the first enclosure, latrines held a large proportion of the total number of faeces deposited (dispersion index, $\sigma^2/\mu \pm SE = 50 \pm 3$, $n = 15$; e. g. 10% of the open area held 78 ± 1% of the faeces. The largest latrine covered, 1.25 m^2, with mean ± SE faeces in each record ± SE per grid point = 74 ± 5, n = 75). The main latrines were formed in the corners adjacent to the hay stack, although there were some groups of faeces along the side walls (see fig. 3.2). In contrast, the centre of the enclosure, including the heavily used feeding bowls and water pot, were relatively clean (in central areas, 15 m^2, mean number of faeces deposited over 2 days ± SE per grid point = 0.47 ± 0.04, n = 900; food bowls and water pot, 3 m^2, 1.0 ± 0.2, n = 180).

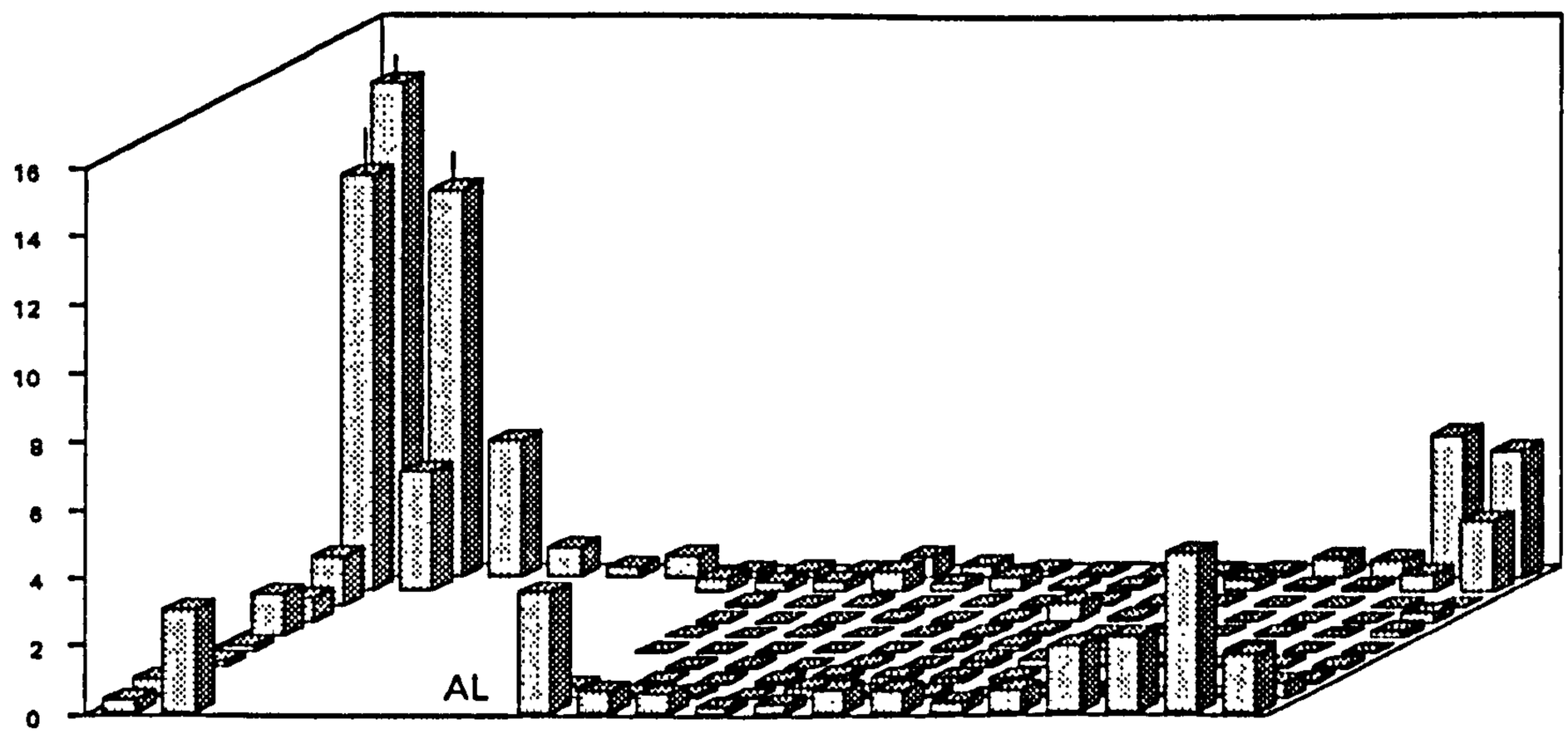


Fig. 3.2 Spatial pattern of faeces distribution. Bars represent the proportions at every location in relation to the total number of faeces (cumulative for every record) recorded in each count. Counts were recorded every two days over a 30 day period (mean percentages; most standard error bars too small to be shown).

The location and the proportion of faeces found in latrines remained stable through time (in fig. 3.2 only a few standard error bars have been drawn because the rest are too small to be seen). Only one latrine (termed AL in fig. 3.2) was apparently abandoned because it was covered with hay as the rats scattered it from the stack.

Latrines were also found in all additional studied colonies where rats deposited a moderate number of faeces outside the nest (see table 3.1; colonies 4 and 5 only deposited a small number of faeces outside the nest). A similar pattern of relatively clean areas around feeding points was also found in these enclosures. Latrines in these colonies always occupied the corners of the pen. The largest latrines were in the corners by the hay stack.

Table 3.1. Faecal dispersion indices in enclosures containing different numbers of rats for different housing periods.

Colony	No of rats	Housing period	Dispersion index σ^2/μ	Remarks
1	17	1 month	50 ± 3	Counts every two days
2	5	7 months	175.87	Final count
3	14	7 months	201.86	Final count
4	1 pair	1 month	7.67	Final count
5	1 male (the female died)	1 month	3.38	Final count
6	1 pair	3 weeks	43.69	Final count
7	1 pair	3 weeks	47.75	Final count

3.2.5. Discussion.

Wild rats, like some other species of mammal, appear to form latrines. The small variability in the proportion of faeces held in

latrines indicates that they are stable through time and that rats deposit faeces at them continuously. However, it does not follow from this that latrines play a role in chemical communication. Rats may be visiting latrines just because corners offer more shelter than other areas of the enclosure. However, Eilam and Golani (1989) found that rats prefer to stay in corners and along boundaries even in an arena with no walls, despite the fact that, in this case, such areas do not offer protection. They also found that rats investigated the substratum more at these sites (perhaps because rats prefer to deposit faeces and urine at these sites, although these researchers did not measure scent marking). Furthermore, the fact that feeding areas, where rats spent a great deal of time, did not hold a great proportion of faeces suggest that they are not the result of incidental deposition. In other species, such as hippopotami, individuals build latrines mainly in areas with some cover (bushes) whereas they seldom form latrines in feeding areas (Klingel, personal communication). Rabbits seem to clear grass off small patches where they build up latrines (Bell, 1980; these bare ground areas occupy an area about the size of an A4 sheet, personal observation), which again dissociates latrines from feeding areas.

The fact that feeding bowls were almost devoid of faeces seems to contradict Laland and Plotkin's (1991) finding that faeces around food bowls add to other olfactory cues to influence social learning. The apparent contradiction regards the suggestion that rats leave cues for social learning *at* feeding points, whereas they did not do so in my study. In their study Laland and Plotkin (1991) constrained the movements of a demonstrator rat to one arm of a U-shaped cage. The rat deposited faeces and presumably other scents around the food bowl. The reason why in my study no rat

faeces were found at feeding points may be that they deliberately avoid depositing faeces at such areas, or perhaps rats ate faeces at feeding points in preference to those elsewhere. However, the instances where coprophagy was observed did not occur at the food bowls. Another alternative may be that whereas laboratory rats are not concerned about predation risk, wild rats might be very sensitive to it. Thus, wild rats might try to keep faeces far from feeding points, especially if only a few sources of food are available, in order to avoid leaving cues to predators. This finding does not necessarily contradict Laland and Plotkin's (1991) interpretation of the effect of faeces on social learning because, in wild rats, faeces may still influence diet preferences even if they are not found at feeding points.

Because only faeces deposited in open areas were recorded, this study cannot assess a possible preference that rats may have for depositing faeces in the hay stack. A density of faeces apparently higher than in most open areas could be detected on the hay. However, many of them may have been buried in the hay as the rats dug their burrows. It is not obvious what sort of bias this may have produced in the observed distribution pattern of faeces in open areas. If rats had a preference for depositing faeces in the hay, it should be expected that they would not deposit them in areas nearby because rats would probably travel a short distance to deposit them on the hay stack. A greater density of faeces should be then expected in areas far from the nest. However, the opposite effect would be produced if some of the faeces fell from the hay onto nearby areas. This seems unlikely because hay constitutes a rough substratum where faeces do not easily slip down. If, on the other hand, rats preferred to deposit faeces outside the hay stack, two

patterns may arise: if they only travelled a short distance to deposit faeces the density around the nest would be greater than elsewhere. If they avoided leaving faeces nearby the nesting areas a greater density of faeces would be expected in the areas furthest from the hay stack. In both cases, unless some factor not considered here biased the distribution, faeces would be expected to be deposited in a random pattern near or far from the hay stack (according to each hypothesis), and not particularly accumulated at corners. The finding that rats deposit faeces at corners forming latrines might suggest that these areas are sites for information exchange.

Rat latrines appear not to have been referred to previously in the literature (only Calhoun (1962) observed clusters of faeces which he thought were the result of incidental deposition). Although this lack of reference to latrines looks striking in view of the distribution pattern of faeces I observed, it might be the result of hygiene regulations in laboratory studies (cages are usually cleaned every few days to avoid diseases, and the rapid accumulation of faeces and build up of urea and ammonia in such small spaces). Most studies in the wild are concerned with sexual and aggressive behaviours and only marginally with scent marking (Telle, 1966; Robitaille and Bovet, 1976; Boice, 1977). Furthermore, faeces are dark in colour and very difficult to detect against a background of litter. Once faeces are squashed into an homogeneous layer by rats, as they were in pens, they may be detected easily by the rats using olfaction but become very difficult for humans to detect by sight.

In the field, I have observed latrines in a natural infestation of wild rats living on a cattle farm (in Aldsworth, Gloucestershire, UK). In this case, several groups of scattered faeces were found in food stores but these scarcely resembled the latrines found in the

enclosures. Faeces were more scattered and in a more uniform pattern than in the pens which suggested that they may have been formed by the labour of the staff in the stores. However, a distinct latrine was found in the farmer's garden under a cypress tree. Several rat paths cut across the grass which connected nearby trees. Two cypresses were inspected because both had very low branches (at a height of less than 50 cm from the ground) that may have served as shelter, although a latrine was found under one of the trees only. Other trees were not likely to give shelter for the rats and observed from a distance did not seem to accumulate faeces.

3.3. Comparison between number of faeces deposited and time spent in a site.

3.3.1. Aims.

Having established that latrines were a consistent feature of the rat colonies studied (and possibly also of rats living in the wild), it was important to test whether latrines were a result of incidental deposition, or whether rats deliberately deposited faeces at them to serve some purpose. The first possibility (Calhoun's (1962) explanation for the clusters he observed) seemed unlikely because latrines were never found in the proximity of the feeding areas, where rats spent a substantial proportion of their time. To assess the existence of a correlation between the proportion of faeces and the amount of time spent in an area, the time rats spent in latrines was compared with that in other areas frequently used by rats. A lack of match between time and number of faeces would suggest that the formation of latrines may have some function.

3.3.2. Methods.

The activity of the rats was video-recorded during the mapping period in the large rat colony. Recording sessions lasted two hours a day during the period of maximum activity (2200 to 2400 h; Calhoun, 1962; Barnett, 1975; Nieder, 1985; and personal observation in these colonies). Each 2 h session was divided into 8 periods of 15 minutes which constituted blocks in the analysis. The sites monitored were:

-the two main latrines (referred in the text and figures as L1 and L2), monitored for four days;

-both feeding bowls (referred as LFB and RFB), monitored for four days;

-the water pot (referred as WP), monitored for two days to increase the sampled sites with the resources available;

-a quarry pipe in a drainage channel (section 2.3) which was frequently used by rats because it provided the only cover in open areas (referred as DP). This site was also monitored for two days for the same reasons mentioned before.

The areas monitored were approximately 1 m^2 for each feeding bowl, the same area for the water pot, and also 1 m^2 for each latrine. The length of the drainage channel monitored was also 1 m, but its area was about 0.15 m^2. The frequency and duration of each visit was transcribed from the video tapes. The duration of a visit was computed as the time spent by the rat whilst in the area monitored.

Because the hypothesis to be tested was whether the accumulation of faeces at latrines was due to incidental deposition,

only the total time spent by rats in each site was used. Individual identity was not used and the variable computed was the number of 'rat units of time', i.e. the number of rats present per session and the time each one spent at each site. Thus, the rate of deposition of faeces was considered similar and for example, 10 rats stopping for 1 minute each at a site were considered to produce as many faeces as 1 rat stopping for 10 minutes at the same site.

Because Calhoun's incidental deposition and the deliberate deposition hypotheses gave precisely oppossite predictions, and therefore, were not independent, only the deliberate deposition hypothesis was tested (as this seemed to correspond to my a priori by observations), using a specific-design one-way non-parametric ANOVA for unequal cell frequencies (Meddis, 1984).

A non-parametric test was chosen because non-parametric tests do not make any assumption about the distribution of the data. The predictions were as follows:

-Deliberate deposition hypothesis. Rats visit latrines to deposit faeces, and perhaps to investigate them. Rats spend a long time at feeding areas (food bowls and water pot) and presumably, also at the drainage pipe because they stop frequently. In contrast, they spend less time in sites visited only to deposit and investigate faeces such as latrines. Thus, the expected rank of time spent at each point should be:

$$\frac{L1+L2}{2} < \frac{LFB+RFB+DP+WP}{4}$$

Or, expressed as coefficients:

$$\lambda d = -2L1, -2L2, +1LFB, +1RFB, +1DP, +1WP$$

Where L1 means time spent on main latrine, L2 on latrine by the wall, LFB and RFB on left and right food bowl respectively, DP, on drainage pipe by a channel and, WP, on water pot.

3.3.3. Results.

The hypothesis that rats spent less time at latrines that at non latrines sites was highly significant ($Z=5.779$, $p<0.001$). The corollary of this is that Calhoun's (1962) hypothesis, that rats defecate at a roughly constant rate in all sites, could not be true. Rats spent more time in relatively clean areas (food bowls, water pot and drainage channel) and seemed to visit latrines to deposit faeces, as shown in Fig. 3.3.

3.3.4. Discussion.

As previously suggested by direct observation, the number of faeces found in an area did not match the time that the rats spent there. Rats spent more time in areas almost devoid of faeces (like food bowls), than in the latrines. Although this analysis was carried out only in one colony, the fact that food bowls remained relatively clear of faeces in all colonies corroborates the idea that rats do not deposit more faeces the more time they spend in an area.

However, a lack of correlation between the time rats spend in a place and the amount of faeces they deposit at it does not necessarily imply that rats deposit faeces at latrines deliberately. They might defecate at corners because they are more scared in these locations. Many authors have suggested that defecation is a result of fear (Harkins, Becker and Wright, 1974; Gentsch, Lischtsteiner and Feer, 1981; Genstch et al., 1982; Viveros, Hernández and Gallego, 1990). But it is very unlikely that in

partially sheltered areas like corners rats were frightened whilst they were not in completely unsheltered areas like feeding bowls (or other

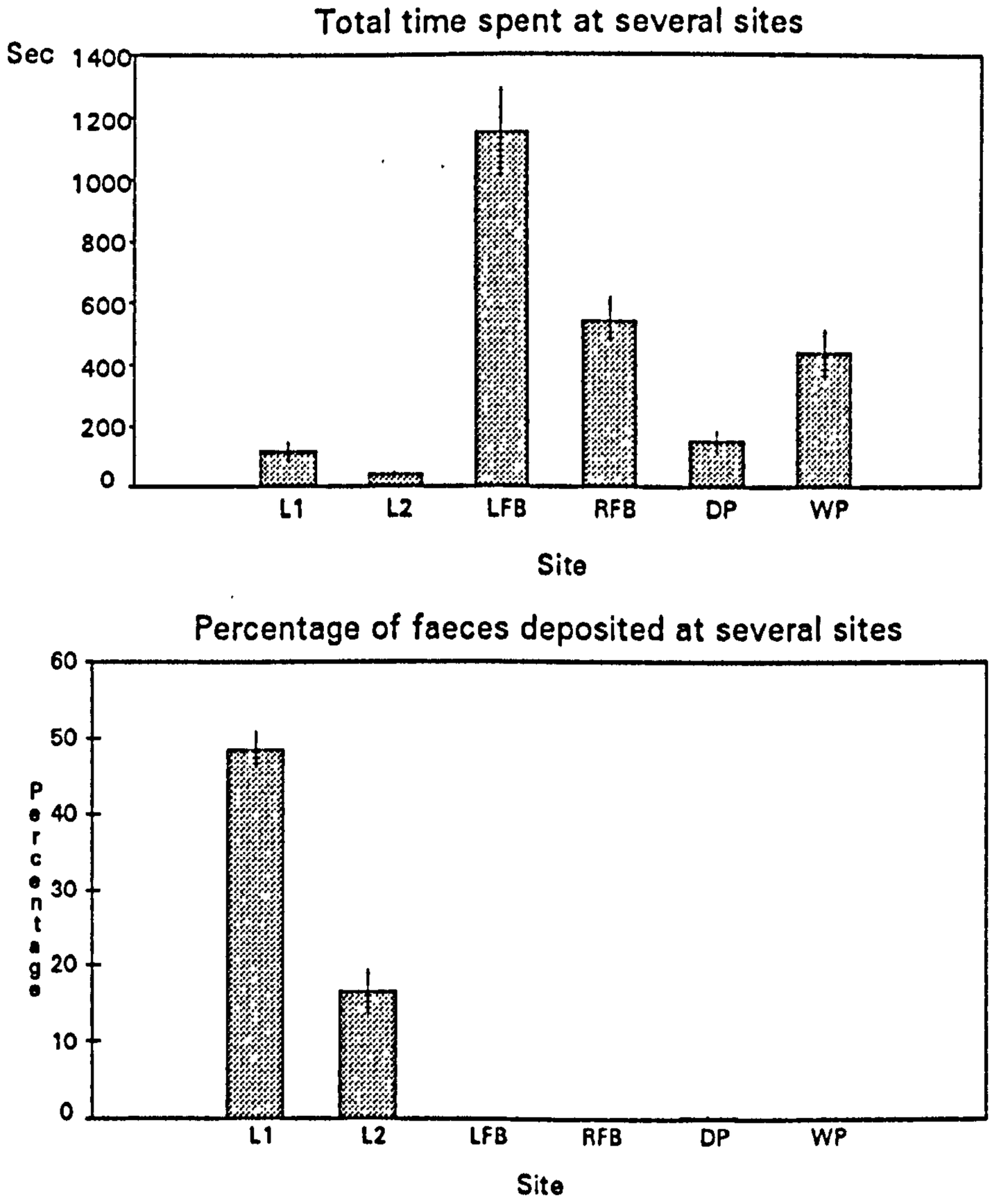


Fig. 3.3. Time spent (upper graph) and faeces deposited (bottom graph) by rats at two latrines and four other sites of similar area frequently visited (mean ± SE). The bottom graph shows the percentage of the total number of faeces deposited in open areas that were found in the monitored sites during the period the rat activity was recorded. L1 and L2, main latrines in the enclosure; LFB, and RFB, left and right feeding bowls, respectively; DRP, Drainage pipe by a channel; WP, water pot.

open areas of the enclosure). Latrines, thus, appear to be created for some purpose. This might be either for hygiene or communication, or a mixture of both.

This is apparently the first case in which latrines have been reported in wild rats. In other species, latrines seem to play a role

mainly in aggressive advertisement (e.g. rabbits, section 3.1; badgers: Roper, Shepherdson and Davies, 1986; Roper et al., 1993; hyenas: Gorman, 1990; hippopotami: Klingel, 1991; rhinoceros: Owen-Smith, 1971). However, faeces may also be placed in a specific site to avoid risk of infection as, for example, may be the case in the aboriginal house mouse (Hurst and Smith, 1995).

3.4. Can rats obtain odour cues from faeces?

The finding that wild rats deliberately form latrines and the widespread use of latrines in communication found in the literature suggests that rats may use faeces to broadcast information. Two roles that rat faeces seem to play in communication have already been indicated: they appear to carry information about diets, and faeces from lactating females attract pups. However, no experiments appear to have examined whether faeces from non-lactating females play a role on their own in communication.

The next experiment was aimed to test whether rats could discriminate between faeces from different sources including themselves. Rats were offered a choice between faeces from known donors and fresh control faeces collected from their own pen. Investigation of these stimuli by the rats in their home pen was recorded to assess the interest of males and females in each type of stimulus. A differential response would imply discrimination probably based on chemical cues (i.e., assuming that faeces from one individual do not look different to those from other individuals). However, as pointed out by Waldman, Frumhoff and Sherman (1988), a lack of discrimination would not demonstrate that rats do not detect and use chemical information because rats may simply

not have the motivation to investigate some odours for longer than others, even if they can differentiate them.

Although absence of discrimination would not mean that faeces play no role in communication, a significant response would suggest that rats can use information conveyed by faeces and that faeces are likely to be used in communication. This experiment cannot discriminate between hygienic and communicatory hypotheses for latrines because, on the one hand such functions would not be mutually exclusive and, on the other, the finding that faeces played a role in communication does not necessarily imply that latrines have a communicatory function. However, examination of any differences in investigation shown between stimuli may give an indication of the possible role of faeces in communication, by comparison of the response towards faeces from males and females, or from residents and other colonies.

3.4.1. Aims.

-To find out whether rats can discriminate between faeces from different donors, particularly whether they could discriminate between faeces from males and females and among different males.

-To assess the possibility that faeces play a role in communication. A communication role for latrines and the possible existence of faecal marking might thus be suggested.

-To assess the rank of investigatory differences towards donors of differing sex and familiarity and thus, suggest possible communicatory roles for faeces.

3.4.2. Methods.

Four pairs of adult rats were used for this experiment (colonies 4 to 7; section 2.5). The rats had been housed in their pen for more than one week to habituate before the experiment began. All the animals survived except one female, which was discovered dead in a nest box a few days after her release. The cause of death was unknown. Because the analysis tested male and female differences, the data from this colony was discarded and only the responses of the remaining 3 colonies were analysed.

The rats were kept individually in cages prior to their transfer to the large enclosures in order to collect faeces for the experiment (section 2.6). These were collected each day, sealed in plastic sample bags containing two or three faeces (depending on size) and immediately frozen to keep their composition as close as possible to fresh faeces. Rats seemed very sensitive to the experimenters while individually caged and frequently squeaked and chattered their teeth. This suggested that they were scared and stressed because of the researcher's presence. As discussed in section 3.4.4, this might have influenced the results obtained.

The experiment consisted of a choice test between faeces from a known donor and faeces from the pen as controls. Experimental faeces were collected as described above from the following donors:

-Resident male (resident indicating subject of the experiment).

-Resident female.

-Neighbour male (that living in the neighbouring pen to which the subjects had olfactory access through a pipe described in section 2.3).

-Unfamiliar male.

Faeces from resident male and female were used as a control to compare the response of each resident to unfamiliar and neighbour faeces with the response to own faeces collected under similar conditions. In addition, comparing the residents response to own faeces and those of their partner might show whether rats can discriminate the sex of the faeces donor.

Fresh faeces (judged by their moist and bright surface) were used as controls. These were collected every night immediately before the start of a trial from the top or surroundings of the hay stack to act as controls. Thus, controls were a mixture of faeces from resident male and female in an unknown proportion. It was impossible to discern to whom they belonged by sight.

Faeces were placed on either of the tiles sited at the corners closest to the hay stack. These sites were chosen because they were surrounded by latrines and rats were seen to approach them to investigate faeces. The tiles used were the same every trial because they were heavily urine marked and exchanging them for clean ones every night might have had an unexpected influence (see section 4.7). Placing faeces from other colonies on the tiles may have influenced subsequent trials, which shall be discussed in section 3.4.4. Four to six faeces (depending on their size) were placed on each tile. Rats were habituated to the tiles because thirty eight tiles had been placed in the enclosure for different experiments (section 2.6).

Six replicate trials per faecal donor and enclosure were conducted. Both experimental and control faeces alternated between corners in successive trials and the order of presentation for different donors was randomised. Experimental and control faeces were marked with a small white dot using liquid paper so that they

could be recognised and discarded after each trial. The activity of each individual at both corners was recorded on videotape for eight hours every night (2100 to 0500 h), using infrared cameras connected to a four-way splitter and this, in turn, to a time-lapse video recorder (section 2.6). Each camera monitored an area of approximately 1 m^2.

The following variables were measured for analysis: total time per trial in monitored area, mean time per visit to monitored area, number of visits to the tiles, total time per trial on the tile presenting the stimuli, mean time per visit to this tile, number of faecal manipulations (instances in which individuals manipulated faeces with their forepaws), and the number of faeces deposited.

Analysis of investigatory responses. Tests measuring investigatory behaviour examined whether rats could discriminate between faeces from the resident male and female, and also whether they could discriminate between faeces from different male donors (resident, neighbour and unfamiliar male). To reduce the confounding effect of comparing sets of responses (towards the experimental tile minus the response to the control tile) differing in their duration but not in their relative preference or avoidance towards either type of tile, the logarithm of the time involved in investigation was used for analysis. Thus, the tests involved proportions of time rather than their absolute value.

A set of general-design two-way non-parametric ANOVAs (Meddis, 1984) tested for the effect of day and sex on each variable. Data were ranked within pens to take into consideration the non-independence of repeated measures and to compare male and female responses.

If no habituation effect, nor interaction between sex and day was found, the following analysis examined the mean response of each rat to each type of treatment. Two general-test non-parametric ANOVAs examined the following responses:

-Male versus female response to faeces from the resident male and female.

-Male versus female response to faeces from the resident male, the neighbour male and the unfamiliar male.

Again, data were ranked within pens to compare the male and female responses to the treatments involved.

Tests involving manipulation of faeces. A Mann-Whitney U test was performed on the number of faecal manipulations by males and females to assess any sex bias in this behaviour. A one-way non-parametric ANOVA assessed the difference rats showed in the manipulation rate of control versus experimental faeces. In both cases the data from all treatments were pooled due to the small size of the sample.

3.4.3. Results.

No day effect nor interaction between day and sex was found for any of the variables or treatments tested (see table 3.2). Therefore, means were computed for each rat and treatment to compare responses towards different treatments.

Rats discriminated between faeces from themselves and their opposite sex partner in respect of the following variables (see table 3.3 for this and the following discussion): mean time on tile per visit ($H_{1df} = 8.80$, $p<0.01$), total time on tile per trial ($H_{1df} = 6.11$, $p<0.05$), but not in the total time spent in the monitored area per trial ($H_{1df} = 0.24$, ns), nor the number of visits to tile ($H_{1df} = 0.05$).

Males and females responded similarly, showing a greater interest in experimental faeces compared to control ones when the experimental faeces belonged to the resident male than when they belonged to the resident female, and no interaction term was significant (mean time on tile per visit, sex effect, $H_{1df} = 0.00$, ns, interaction term, $H_{1df} = 2.20$, ns; total time on tile per trial, sex effect, $H_{1df} = 0.98$, ns, interaction term, $H_{1df} = 0.98$, ns; total time on monitored area per trial, sex effect, $H_{1df} = 0.98$, ns, interaction term, $H_{1df} = 0.00$, ns; number of visits to tile, sex effect, $H_{1df} = 0.98$, ns, interaction term, $H_{1df} = 0.00$, ns).

Table 3.2. Effect of day and sex in the rat response towards faeces from different donors. ln means napierian logarithm.

Variable	Faeces donor	Day		Sex		Interaction	
		H_{1df}	p	H_{5df}	p	H_{5df}	p
ln of mean time on tile per visit	resident male	4.86	ns	0.14	ns	4.32	ns
	resident female	6.19	ns	1.24	ns	3.28	ns
	neighbour male	2.85	ns	0.42	ns	7.05	ns
	unfamiliar male	4.64	ns	0.12	ns	7.70	ns
ln of total time on tile per trial	resident male	2.99	ns	0.08	ns	7.97	ns
	resident female	6.04	ns	0.06	ns	2.86	ns
	neighbour male	6.28	ns	0.11	ns	6.76	ns
	unfamiliar male	3.75	ns	5.92	0.05	5.03	ns
ln of total time in monitored area	resident male	2.19	ns	0.20	ns	4.67	ns
	resident female	3.18	ns	0.05	ns	3.66	ns
	neighbour male	10.80	ns	1.42	ns	4.03	ns
	unfamiliar male	5.08	ns	4.45	0.05	4.28	ns
number of visits to tile per trial	resident male	4.02	ns	1.99	ns	8.99	ns
	resident female	4.88	ns	0.79	ns	4.57	ns
	neighbour male	6.28	ns	1.78	ns	4.35	ns
	unfamiliar male	3.87	ns	1.67	ns	6.73	ns

Rats also discriminated between faeces from different males (themselves, neighbour and unfamiliar, comparing the investigation

of experimental versus control faeces, see table 3.3) in the total time on tile per trial (H_{2df} = 9.82, $p<0.01$) and number of visits (H_{2df} = 9.10, $p<0.05$), but not in the mean time on tile per trial (H_{2df} = 1.97, ns) nor the total time in the monitored area per trial (H_{2df} = 4.64, ns; see fig. 3.4 for these and following results). The difference between the response of resident males and females was statistically significant for the mean time on tile per visit (H_{1df} = 5.38, $p<0.05$) and total time on tile per trial (H_{1df} = 4.05, $p<0.05$), but not for the total time on monitored area per trial (H_{1df} = 0.45, ns), or for the number of visits to the tile per trial (H_{1df} = 0.51, ns). Again, no interaction term was found (mean time on tile per visit, H_{2df} = 5.06, ns; total time on tile per trial, H_{2df} =0.97, ns; total time on monitored area per trial, H_{2df} = 1.76, ns; number of visits to tile per trial, H_{2df} = 3.37, ns).

Table 3.3. Preference of wild rats for faeces of known donor (experimental faeces) over those from their own enclosure (control ones). Positive values indicate a preference for experimental faeces (or avoidance of control ones), whereas negative values show the opposite. The data include the male from colony 5, although this was excluded from the statistical analysis. The acronyms represent respectively, RM resident male, RF resident female, NM neighbour male, UM unfamiliar male, MTO mean time on tile per visit, TTO total time on tile per trial, TMA total time in the monitored area per trial, VIS number of visits to tile per trial, Var variable, S. sex, sex of the subject investigating the stimuli. Time measures are expresed in seconds.

		Donor of experimental faeces								
		RM		RF		NM		UM		
Var	S. sex	Mean	SE	Mean	SE	Mean	SE	Mean	SE	n
MTO	Male	2.35	1.20	0.85	0.17	1.28	0.31	1.88	1.14	4
	Fem.	2.78	1.10	-0.23	0.36	0.86	0.18	2.50	0.50	3
TTO	Male	9.71	2.19	3.65	1.57	1.14	2.31	3.33	1.62	4
	Fem.	9.03	4.90	1.58	1.20	-0.08	0.42	6.31	1.60	3
TMA	Male	159.58	122.84	-76.20	64.33	-78.07	95.89	-43.21	57.94	4
	Fem.	-22.40	39.64	51.88	116.95	-94.00	75.08	2.83	22.93	3
VIS	Male	1.17	0.70	0.68	0.31	-0.71	0.68	-0.1	0.49	4
	Fem.	0.13	0.24	0.13	0.77	-0.81	0.48	-0.11	0.11	3

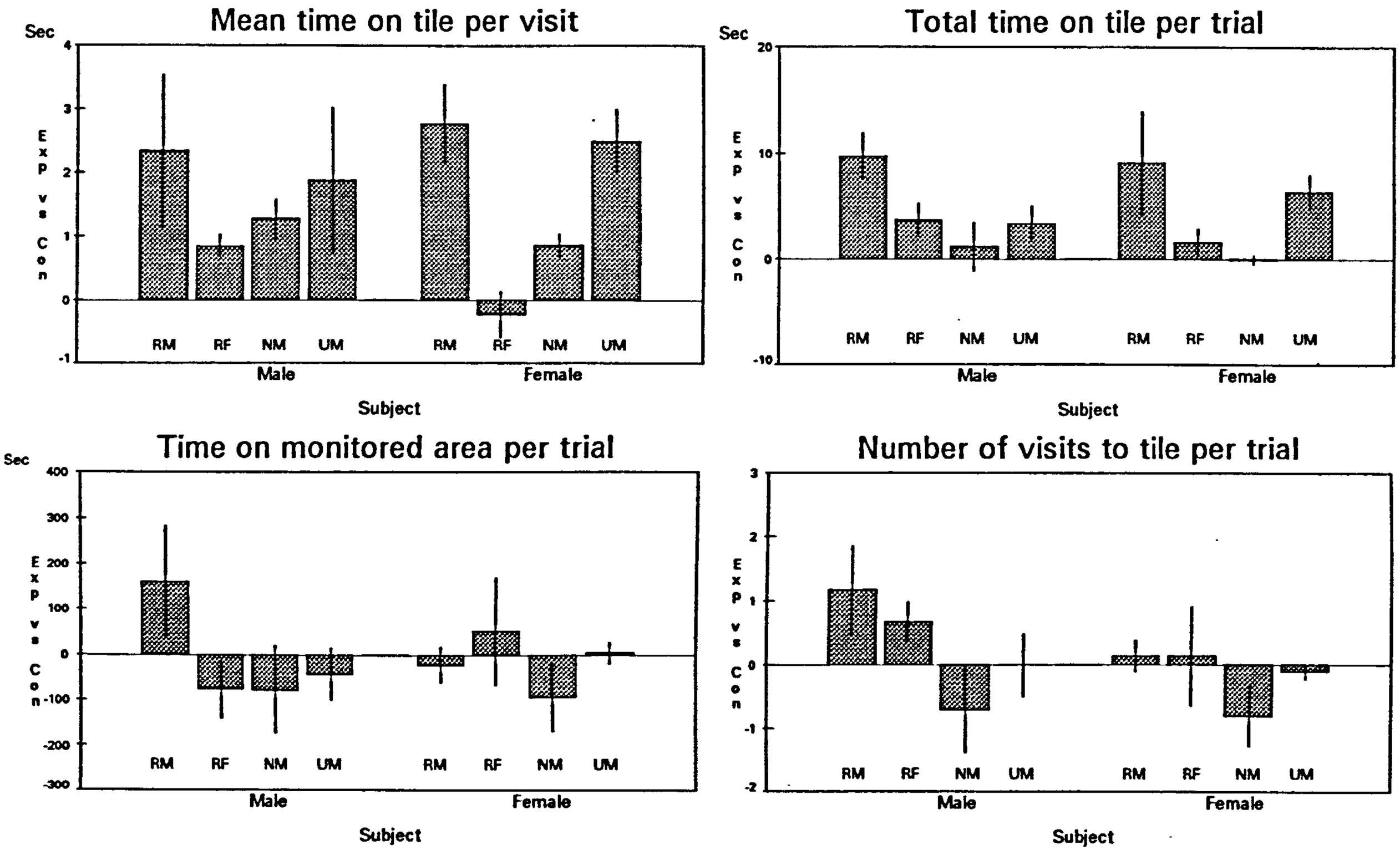


Fig. 3.4 Response of wild rats living in pairs to a choice test between faeces from a known donor (Exp) and faeces from their own enclosure (Con) ($\mu \pm SE$, difference in response to Exp - Con). The acronyms represent, RM resident male, RF resident female, NM neighbour male and UM unfamiliar male vs control faeces.

Although males seemed to manipulate faeces more often than females did, the result did not achieve statistical significance, probably due to the great variability of the data and the small number of individuals involved (Mann-Whitney U test, $H=1$, $P>0.05$; mean of all faecal manipulations recorded per rat summed across treatments including experimental and control faeces ± S.E., males=22.75 ± 7.47, n=4; females=3.06 ± 1.76, n=3). Rats did not prefer manipulating the experimental faeces compared to control ones (one-way non-parametric ANOVA, $H=1.33$, $p>0.05$).

3.4.4. Discussion.

The results showed that rats could discriminate between faeces from different donors. Because experimental faeces looked very similar to the researcher (although they were softer and moister than control faeces), the most likely explanation for this response is that rats discriminated on the basis of chemical cues. However, the possibility that rats used some sort of visual or tactile cue undetected by the experimenter cannot be ruled out.

Rats discriminated between faeces from the resident male and female. The results from table 3.3. show that experimental faeces attracted more attention than control ones. In addition, rats showed more interest towards experimental faeces from the resident male than towards those from the resident female. This is striking because the control faeces consisted of an unknown mixture of male and female faeces. There are several possible explanations for these results.

Resident males and females may have recognised the faeces as belonging to themselves. In such case, the interest towards the experimental faeces might have been greater than towards the

control faeces because the method of collection made the experimental faeces less familiar than the control ones (see a list of such factors below). The discrimination between faeces from resident males and females can be explained if faeces are used as a status badge, as urine is in mice (section 1.3.2.2.1.1). In such case, any changes affecting the dominant male should be more interesting for both resident rats than changes affecting individuals lower in the social rank (the resident female in this case, section 1.4.5.1).

Some of the factors regarding the method of collection of experimental faeces or the experimental design may have made the experimental faeces unrecognisable as faeces from the own colony (see below). Thus, the results would be showing not the differential response to faeces from the own colony, but from male and female individuals identified by the residents as unfamiliar rats. If rats discriminated between such unfamiliar male and female individuals, rats would be showing their ability to recognise gender from faeces. Experimental faeces from the resident male attracted more attention than those from the resident female. If rats did not recognise the experimental faeces as theirs, this might show a greater interest for unfamiliar males over unfamiliar females. Although this might suggest gender recognition, the results are not conclusive because rats may have been discriminating between experimental faeces in terms of some chemical factor that was more attractive or was produced in larger quantities by individually caged males than by individually caged females, without showing such sex bias in natural situations. For example, rats might produce in their faeces some stress factor (see below). If males are more easily scared than females male faeces would attract more attention than female ones. Alternatively, such factors may provoke avoidance and be produced

in greater quantities in female faeces. This might be supported by the fact that both sexes showed a similar response. Such similarity might also be explained if the faeces were not recognised as those of the own colony: Because individuals of the same sex tend to compete more than those from opposite sexes, an unfamiliar male might pose a greater threat than an unfamiliar female for the resident male. Because female rats were usually pregnant, the similar response shown by resident females might be due to the danger than an unfamiliar male might commit infanticide on her offspring. As mentioned in section 1.4.5.1., Calhoun (1962) found that pregnant and lactating female wild rats tend to exclude conspecifics from their nesting areas, while Albert et al. (1988) found female aggression to be greatest towards intact males, lowest towards females. However, results from naturally deposited marks discussed in sections 3.5 and 3.6 show a different trend.

Rats discriminated between faeces from different males. These results show that rats could use cues from faeces to discriminate between different individuals of the same sex. However, as Halpin (1986) pointed out, this does not mean that rats can recognise individuals but only individual odours. Again, the results seem surprising because faeces from the resident male attracted most attention, whereas those from the neighbour male attracted least. If faeces were used in territorial marking, advertisement of dominance or some other role in inter-male competition, the resident male should investigate faeces from individuals from other colonies more than his own. According to Fisher's dear enemy hypothesis (see review in Temeles, 1994), because unfamiliar males pose a greater threat to the resident male than a neighbour male, the resident male should investigate the

faeces from an unfamiliar male more than those from a neighbour. This would only happen provided that the resident rats could recognise the rats from the pen adjacent to theirs as neighbours. This result was supported by the data. However, the great interest shown towards faeces from the resident male is very difficult to explain in terms of communication between competitors. Perhaps two factors may have affected the response of the rats at the same time: on the one hand the resident male and female may have responded with strong investigation of the faeces of the resident male because they conveyed some important change in the health/stress status of the top individual, whereas the response towards faeces belonging to males from other colonies showed their interest in intruders of different familiarity. The reason for a different response of resident males and females in this context (contrasting with their similar response in the previous comparison) would show the greater involvement of resident males in defending the territory (section 1.4.5.1). Alternatively, these results might be explained in terms of social novelty without considering inter-male competition: faeces from own colony males might receive increased attention because they constituted a familiar stimulus slightly changed because of the collecting conditions, whereas faeces from the other males would be recognized as novel and therefore investigated more than controls but less than the modified familiar faeces from own colony. However, as in the previous comparison, these results should be interpreted with great caution, as they might be due only to responses towards factors indicating stress or fear arising when the rats were individually caged (see below).

Finally, male rats showed a rate of faecal manipulation slightly greater than females but this was not statistically significant. This

might be due to the small size of the sample involved, or because responses to faeces from different types of donor were pooled together. Further data are needed to discern whether there is a sex difference in faecal manipulation or whether manipulation varies towards different types of donor.

Factors that might have altered the response of the rats. Although the response obtained might be similar to that obtained if the experimental faeces had been deposited by the rats themselves, it cannot be ruled out that a number of factors produced an artifact response of rats towards the introduced faeces. The following paragraph will discuss such factors:

-The method of collection for control and experimental faeces. Whereas control faeces were deposited by free-ranging rats, experimental faeces were collected when rats were individually caged. Cues arising from the stressful method of collection for experimental faeces might have overridden the messages carried by faeces deposited by free-ranging rats. As previously discussed, the rats seemed very sensitive to the presence of humans when caged. They took little food during this period, often squeaked and chattered their teeth and, in some instances, faeces appeared lighter and smaller than those collected from the pens. Brown (1995) suggested that diet, genes and bacteria could interact to produce a unique individual odour. This odour might also be altered if the rat reduced food intake significantly. Although there is controversial evidence suggesting that some scents in rats indicate stress of the donor (section 1.4.7.2.9), faeces do not seem to convey such message (MacKay-Sim and Laing, 1981a). Alternatively rats might be able to manipulate the amount of anal gland secretion they release with faeces, as rabbits do (Sneddon, 1991). Thus, control and

experimental faeces from the same individuals might differ in the amount of anal gland secretion and in the information they carry. A further possibility is that the differences between the anal gland secretion of control and experimental faeces was biochemical. Davies, Lachno and Roper (1988) found such differences in the anal gland secretion of wild and captive populations of badgers.

This effect appeared to be unavoidable. Although great care was taken not to disturb the rats when caged, there appeared to be no alternative to obtain faeces from known wild rat donors other than caging the wild rats individually before releasing them. They could not be kept individually in large enclosures because there were not enough enclosures available. In addition, searching for sufficient fresh faeces in a large enclosure was unlikely to have been practicable.

-Other constraints in the design of the experiment might also account for the great interest shown by rats to experimental faeces from the resident male compared to control faeces. Those collected from the enclosure could not be assigned to the resident male or female. That probably resulted in a variability in the response to controls presumably higher than if control faeces from males and females could have been separated.

-Olfactory cues left on the tiles from previous trials may have interacted with cues emanating from the stimulus faeces in some unpredictable manner. As mentioned in the methods section, the faeces were placed always on the same pair of tiles (but alternating experimental and control faeces on consecutive trials). This decision was taken because I had previously discovered that a clean tile constituted a powerful stimulus for the rats and introducing clean tiles might trigger urine marking and greatly disturb the resident's

response towards the faeces. However, the alternative method also had disadvantages. Although experimental and control faeces were marked with a liquid paper dot to be discarded after the trial, the olfactory cues that they left on corners tiles may have lasted long enough to be detected and influence the rats twelve hours later when the following trial started. However, such potential cues may have had little effect because the tiles were heavily urine marked, which might have masked the odours left by faeces from previous trials or, alternatively, fresh faeces might have masked the response of rats to odours from previous faeces (as scent counter-marking in golden hamsters has shown, section 1.3.2.3). It was impossible to predict how control or experimental faeces from a previous trial might affect the following trial.

3.5. Faecal marking in response to urine cues from neighbours and residents.

The finding that rats could discriminate among faeces from different donors suggested that faeces may play a role in communication. However, because of the faecal collection method, the response observed might not be the same as that found in a more naturalistic experiment. In addition, rats may be able to discriminate between different kinds of faeces, but still not use them in scent marking. If faeces are used as scents for communication, some type of scent marking (faecal marking, urine marking, etc.) should be expected towards faeces deposited by other conspecifics or possibly the deliberate deposition of faeces (faecal marking) in response to other social olfactory cues (urine for example). The existence of such faecal marking would demonstrate a role for faeces in social communication.

3.5.1. Preliminary observations.

The response expected appeared when I was conducting an experiment on urine counter-marking (section 4.8). Counter-marking occurs when an individual deposits its own marks over or by those of another animal (section 1.3.2.3). This may serve a number of purposes, among others to mask the marks of the previous individual (Johnston, Chiang and Tung, 1994). Clean tiles were introduced into the rat's home pen on the first night of the experiment and rats readily urine marked them, covering a substantial proportion of their surface. On the following night, rats were offered a choice test between previously clean tiles urine marked overnight by themselves (control tile) and tiles marked by neighbour or unfamiliar rats (experimental tile). The rats not only counter-marked the tiles using urine (especially that bearing alien urine), but they also deposited an unusually large number of faeces (up to 30 per night) next to the tiles marked by neighbours/unfamiliars. This response appeared to be a faecal marking response similar to that found in other species (section 1.4.7.2.11 and introduction of this chapter).

3.5.2. Aims.

The main aim was to assess whether rats show faecal marking in response to urine cues from rats belonging to own or other colonies. If rats defecate near the tiles simply because they stop at them (incidental deposition), a similar number of faeces should be expected on both types of tile. However, if rats defecate in response to some urine cues more than in response to others, it would suggest that rats might be using faeces for communication. A difference in

the number of faeces found by each type of tile might still be explained as incidental deposition due to a preference for investigating some types of urine. However, if the time spent by males and females at a tile does not match their faecal deposition rate, this would indicate deliberate defecation (faecal marking). In this case, a preference for faecal marking urine cues from neighbours or unfamiliar rats compared to urine cues from residents might suggest that faecal marking is aimed mainly at potential intruders, while a preference for marking cues from the own colony might suggest that it is used for intra-group communication. A similar defecation rate at both tiles could be explained by incidental deposition, orientation, or even communication. In the second case, rats may faecal mark both tiles because they present a large proportion of fresh marks compared to other tiles in the pen that bear aged urine. Faecal marking for orientation may perhaps serve to label new objects that are being incorporated into the familiar background. More specifically, a number of hypotheses, most of which are not mutually exclusive, would predict a greater marking response towards the tile bearing marks from individuals of other colonies: i) the most traditional explanation would be that the message is aimed at potential intruders, to dissuade them from entering the resident's territory or to advertise that the area is occupied; ii) faecal marking, especially if the male marks more than the female, may be a form of dominance advertisement aimed at both own colony residents and intruders; iii) it may also serve a role in sexual competition, because discarding or counter-marking scents from other males may both attract potential mates and disuade the resident females from mating with intruders, either by signalling to the resident females that the resident male is of better quality than

the unsuccessful challenger or by masking the marks of the intruder as explained in section 1.3.2.3.

In addition, sex differences in response were also examined as this might give some indication of the role that faecal marking may play in rat communication. The sex bias in urine marking of clean tiles found in an earlier experiment (see section 4.4) showed that males urine marked clean tiles far more than females did. In the current experiment, the stimuli consisted of clean tiles marked naturally by pairs of rats and thus, most urine presumably came from males. If males showed greater interest and faecal marking than females towards stimuli consisting predominantly of male urine, faecal marking may play a role in inter-male competition. However, because the stimuli were a mixture of male and female urine, it is impossible to know whether individuals were responding to the predominant urine (male urine), to the scarcer one (female urine), or to both at the same time. Although the experiment showed the response towards natural marks, interpretation of the response will thus be limited.

3.5.3. Methods.

The experiment was conducted using five colonies, each housing a pair of rats (colonies 6 to 10, section 2.5). The rats had been released at least one week before the experimental period (which included this and other experiments). Colonies 6 and 7 were housed in 10 x 5 m pens. The experiment was replicated using the remaining colonies in the following year, when CSL staff divided these two pens into four 10 x 2.5 m pens. However, it is unlikely that this would have any effects on the experiment.

The experiment consisted of a choice test between two tiles urine marked by different donors: resident rats and neighbour or unfamiliar rats. The night before the trial, two clean tiles (section 2.6) were introduced in each pen, occupying the position of a familiar marked tile next to a wall. The native tiles were placed outside the pen until the trial finished and then they were replaced back in their original positions (i.e., two days after their removal). As shown elsewhere (section 4.3), the rats urine marked the clean tiles readily overnight. Trials were conducted on the following night when one of the two tiles marked by own colony rats was exchanged with another tile similarly urine marked in one of the other pens. This exchanged tile was designated as the experimental tile. The second tile marked by residents remained in the own colony's pen to act as a control. Control tiles were raised and placed back in their position as a control manipulation. If the experimental tile came from a neighbouring enclosure with which rats had olfactory contact via a linking pipe covered with wire mesh (section 2.3 and figure 2.2), the experimental tile was designated as marked by neighbours. This included the exchange of stimuli between colonies 6 and 7, and those between colonies 9 and 10. If it came from a neighbouring enclosure without such olfactory communication the tile was designated as marked by unfamiliars. This included colony 8, which was isolated from colonies 9 and 10. Because the colony serving as the donor rotated in a latin square as explained below (except in colonies 6 and 7 which always served as

donors for each other), the proportion of trials with neighbour urine stimuli as experimental stimuli was 100% for colonies 6 and 7, 60% for colonies 9 and 10, and 0% for colony 8. However, as both neighbour and unfamiliar cues elicited similar responses, both categories were collapsed into one for analysis.

Overnight defecation was measured on both tiles the following morning. Defecation was measured as the number of faeces deposited on the tile or within 0.5 m sides of the experimental or the control tile, which were always adjacent (and, as mentioned in section 2.6, placed 1 m apart). Faeces were only found on the tiles and at either side of a tile because tiles were sited next to a wall and rats almost always sought the relative protection of a wall in their trips (therefore, faeces were seldom found by the side of the tile opposite to the wall or at a distance from the wall). After each trial, these faeces were removed.

In addition, the behaviour of rats on the experimental tile was recorded on video tape to assess the response of each sex towards alien marks. Behaviour was not recorded on the control tile because pilot observations showed no faecal marking of the control tile and resources were limited. The behavioural variables obtained from the video tape were: total time spent on the tile per trial, mean time on tile per visit, total number of visits per trial and number of faeces deposited per trial by the resident male and female. Data from tapes were transcribed by only one observer, but each sequence was repeated more than once to ensure that identity and timing of the

visit were recorded accurately. Visits in which individuals could not be confidently identified were discarded. For undetermined reasons, the female in colony 8 was not identified on any of the video tapes. This might have happened either because the female was rarely active (neither was the male in this colony), or because her fur marks were dim and thus, data from this female were discarded as doubtful identification.

Records were taken on video for 11 h, from 1800 to 0500 h. That is, the trial started before rats became active (about 2000 h; see section 3.3.2, although some activity could sometimes be observed between 1800 and 2000 h).

Because the experimental and control tiles were exchanged with tiles sited along one of the long walls (always that dividing the pens in the 2 pen set), one of the tiles was always closer to the hay stack than the other. Thus, rats were likely to find the tile closer to the nest before the other. For this reason, the position closer/ farther from nest was alternated between experimental and control tiles to avoid site effects. Although this might have been overcome by placing the tiles in parallel to the nest, and hence perpendicular to the wall, rats would have come across the tile nearer the frequently visited wall pathway more often than the other tile, which is likely to have constituted a greater confounding factor. The position of each type of tile was also alternated between the positions of two familiar pen tiles, so that rats could not get used to finding the same stimuli in the same place, or in case experimentally

induced urine or faecal marks left cues that rats subsequently would respond to instead of responding to the stimuli of the current trial. Only two different positions for each tile were used (four in total) due to restrictions in the movement of the camera used for recording activity. A red light was sited 1 m above the midpoint between tiles to increase visibility in the video records.

The experiments consisted of five replicates for each pen (each consisting of overnight marking of clean tiles during the first night and a choice test between marks from own colony and unfamiliar rats during the second one). The complete procedure was carried out by the author in colonies 8, 9 and 10. However, in colonies 6 and 7 all the trials were carried out by an assistant scientific officer of CSL at the end of the experimental period (i.e., during the fifth week). Due to an unfortunate misunderstanding, the number of faeces by each type of tile was not recorded on the morning following each trial. Thus, whereas the data extracted (in all cases by the author) from the video tapes correspond to the five colonies, the comparison between defecation at experimental and control tiles was measured only in colonies 8 to 10. However, because the current experiment was very similar to the experiment measuring urine counter-marking in response to urine from neighbours (section 4.8), and because the data from the faecal marking response were incidentally recorded in the experiment reported in section 4.8, these data can also be used to compare the faecal marking response towards urine from own or other colonies. It is important to note

that, in the experiment of section 4.8, 9 replicates were carried out per pen. It was conducted in colonies 3 to 7, but only colonies 6 and 7 showed some faecal marking response, and indeed, any kind of defecation in open areas. In summary, in colonies 6 and 7, the comparison between faecal marking response towards urine from residents and other colonies was obtained from a 9-replicate test carried at the beginning of the five-week experimental period, whereas the video tapes measuring behaviour at the experimental tile were recorded in a 5-replicate test carried out during the fifth week. Although the residents' faecal marking behaviour could have been recorded from the video tapes, the faecal marking response to the control tiles would be still missing and, in addition, I found that counts of faeces from video tapes were smaller than those recorded in situ (probably because in situ countings recorded faecal marking responses after the video filming finished at 0500 h).

The comparison of faeces found by experimental and control tiles was analysed using a specific-design two-way non-parametric ANOVA with familiarity and day as main variables (Meddis, 1984). For simplicity in the computing procedure and to standardise with the number of data points in colonies 4 and 5, only data for the first 5 replicates in colonies 6 and 7 were used, discarding the rest. The first 5 replicates were used instead of the last 5 or any other combination so that the analysis could detect any effect of habituation. A non-parametric analysis was chosen because it does not require the data to follow a normal distribution. The specific

test examined whether rats faecal mark urine cues from non-residents (neighbour or unfamiliar rats) more than those from own colony across all days. The respective coefficients used for this analysis were:

	Trial (1-5)				
other colonies	+1	+1	+1	+1	+1
own colony	-1	-1	-1	-1	-1

The data corresponding to the recorded behaviour of males and females towards urine cues from rats belonging to other colonies were ranked within each pen. Thus, on the one hand it was possible to take into account the fact that measures on different days were not independent of each other, and, on the other, it was possible to compare the response of males with that of females and to examine whether, as predicted, there was no day effect. These data were also analysed using a specific design two-way non-parametric ANOVA to examine the prediction that faecal marking plays a role in inter-male competition and therefore, that males respond to urine cues from other colonies (belonging mainly to a male) more than females do (the factors tested being the subject's sex and day). The coefficients for male and female responses for the five days were thus:

	Trial (1-5)				
Males	+1	+1	+1	+1	+1
Females	-1	-1	-1	-1	-1

Plate 3.2. Comparison of response to a tile urine marked by rats from other colonies (tile in the middle) and a control tile marked by resident rats (tile at the bottom). The picture illustrates that faecal marking was specific towards the tile marked by non-residents. Notice also the strong urine marking response towards non-resident urine cues.

The same predictions were tested for each of the variables assessed: total investigation time per night, mean time investigating per visit, number of visits, number of visits in which faecal marking occurred and total number of faeces deposited by males and females.

3.5.4. Results.

Rats deposited faeces by the tile urine marked by neighbour/unfamiliar rats almost exclusively ($Z=5.13$, $p<0.001$; number of faeces deposited in response to urine from other colonies, mean ± SE = 15.08 ± 7.55 faeces, $n=5$; number of faeces deposited in response to resident urine marks = 0.04 ± 0.04 faeces, $n=5$; see fig. 3.5 and plates 3.2 and 3.3). The test might have stimulated a chemical contest between colonies, because one colony each year showed far greater faecal marking rates than the others (colonies 6

Table 3.4. Faecal marking rate of colonies 6 to 10 by tiles urine marked by rats from either own or other colonies. The acronym W represents the weight of the resident male. Colonies 6 and 7 were occupied the pens before colonies 8, 9 and 10.

	Non-resident cues			Resident cues			
Colony	Mean	S.E.	n	Mean	S.E.	n	W
6	6.2	3.7	5	0	-	5	343
7	36.2	3.3	5	0	-	5	370
8	1.8	0.4	5	0	-	5	347
9	0.8	0.6	5	0	-	5	313
10	30.4	3.2	5	0.2	0.2	5	575

and 7 were established simultaneously, as were colonies 8, 9 and 10, see table 3.4). The greatest faecal marking in a set of colonies corresponds to the pair with the heaviest male (colonies 7 and 10).

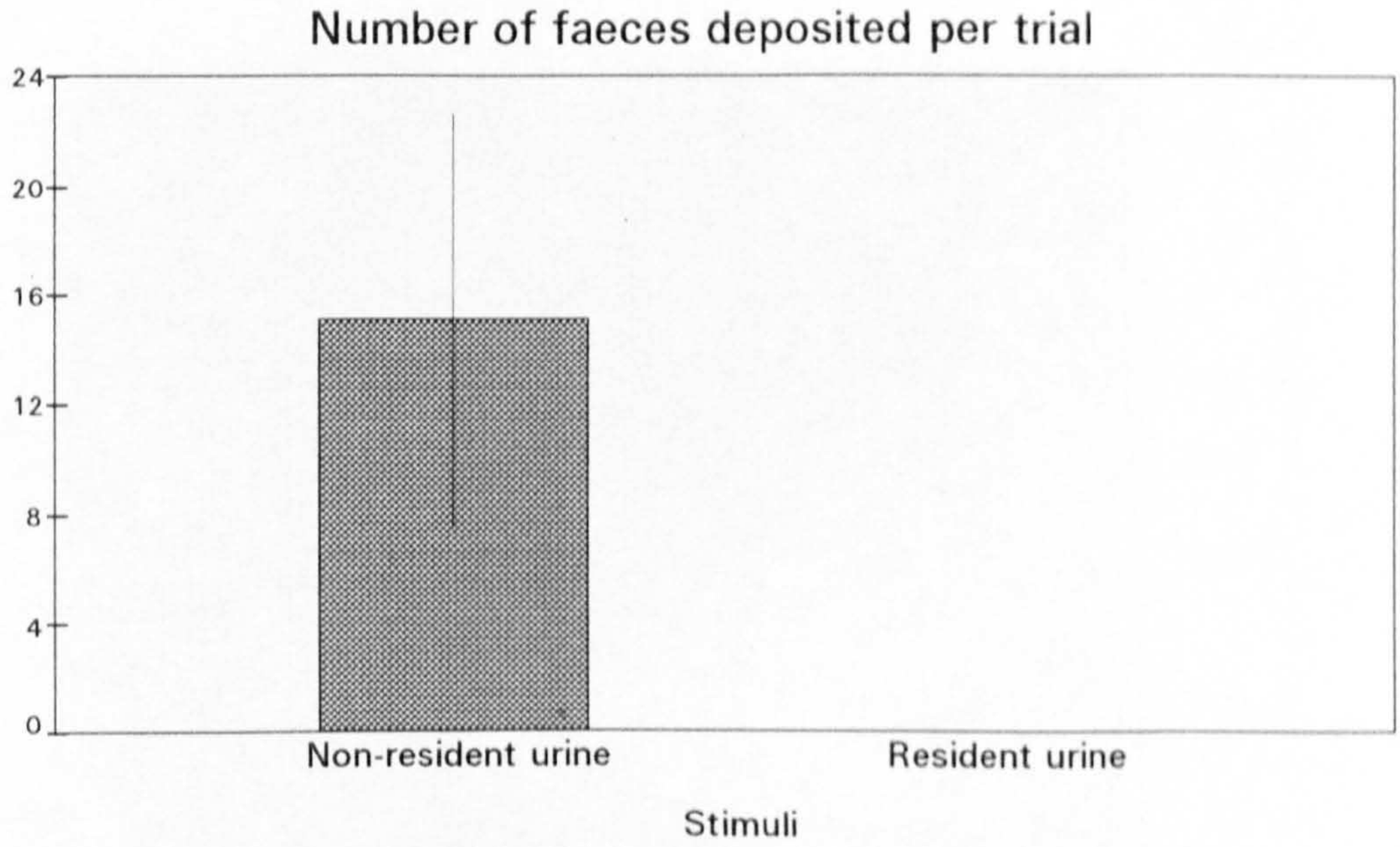


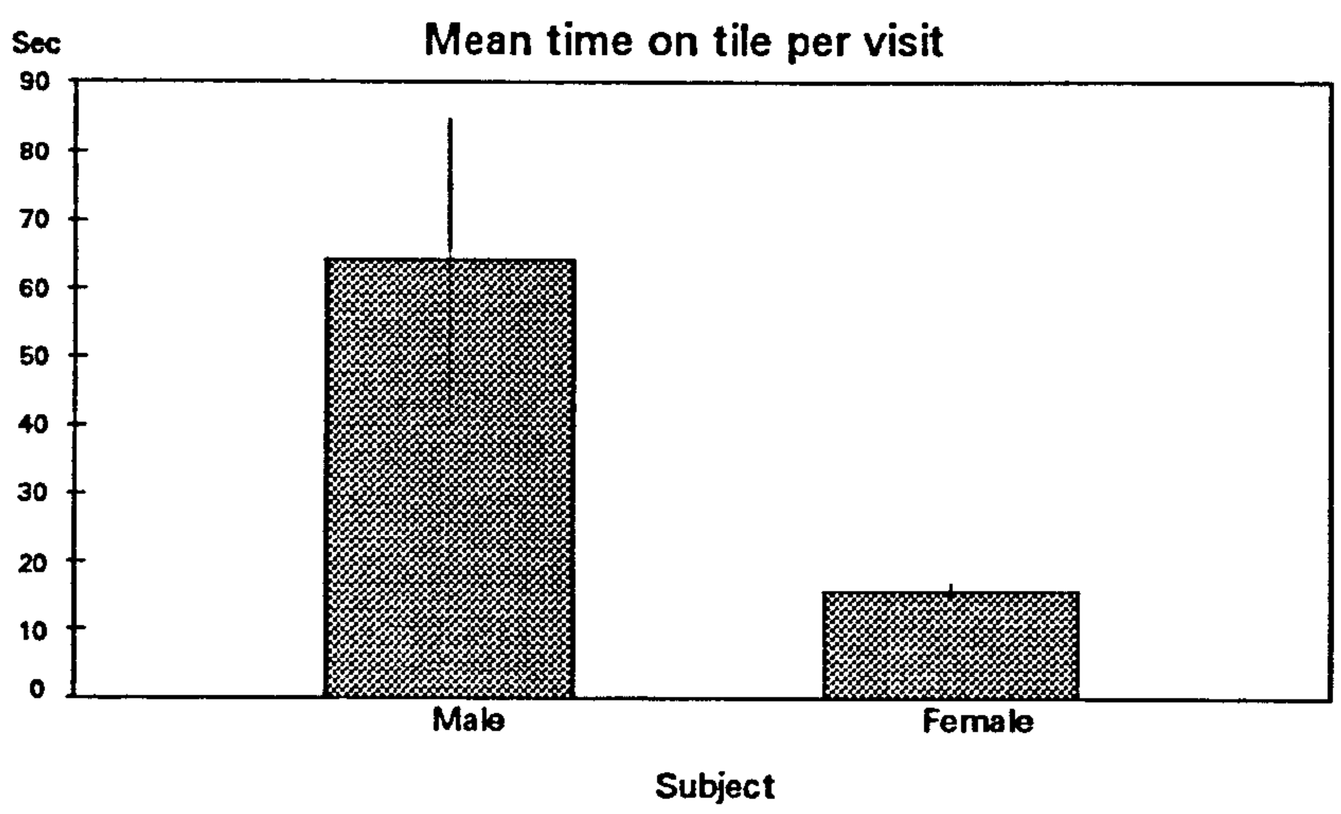
Fig. 3.5 Number of faeces deposited by wild rats in response to tiles urine marked by rats from either own or other colonies. Only males faecal marked (as revealed by monitoring the tile marked by rats from other colonies).

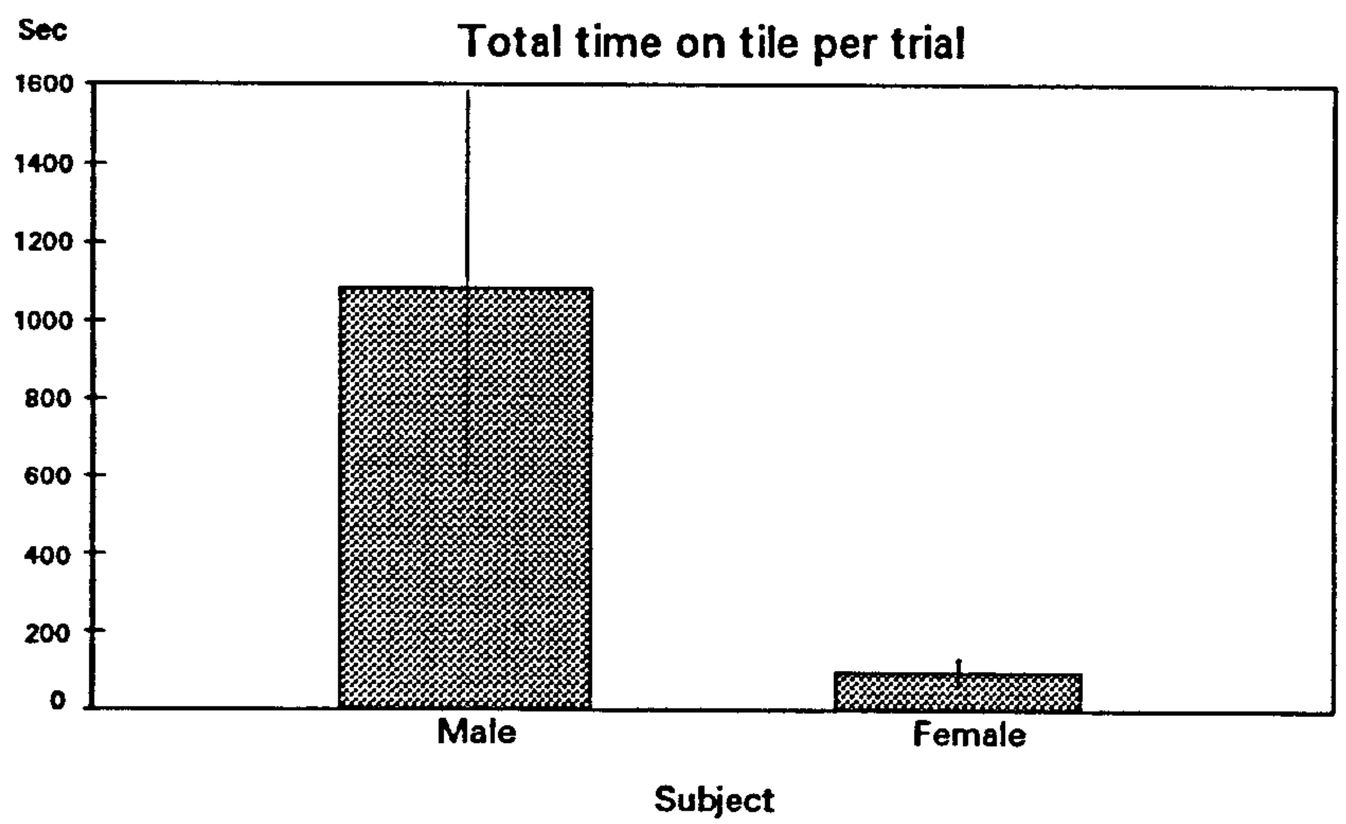
As predicted by the inter-male competition hypothesis, male rats faecal marked and investigated urine from other colonies for longer than females (see table 3.5 and fig. 3.6 for this and following discussion). Only males were observed faecal marking although they only faecal marked in a relatively small proportion of visits (20%). They also visited the tile marked by rats from other colonies more frequently than females did ($Z=2.43$, $p<0.01$) and investigated urine cues from other colonies more both with respect to mean time on the tile per visit ($Z=2.75$, $p<0.01$) and the total time spent on the tile per trial ($Z=2.55$, $p<0.01$).

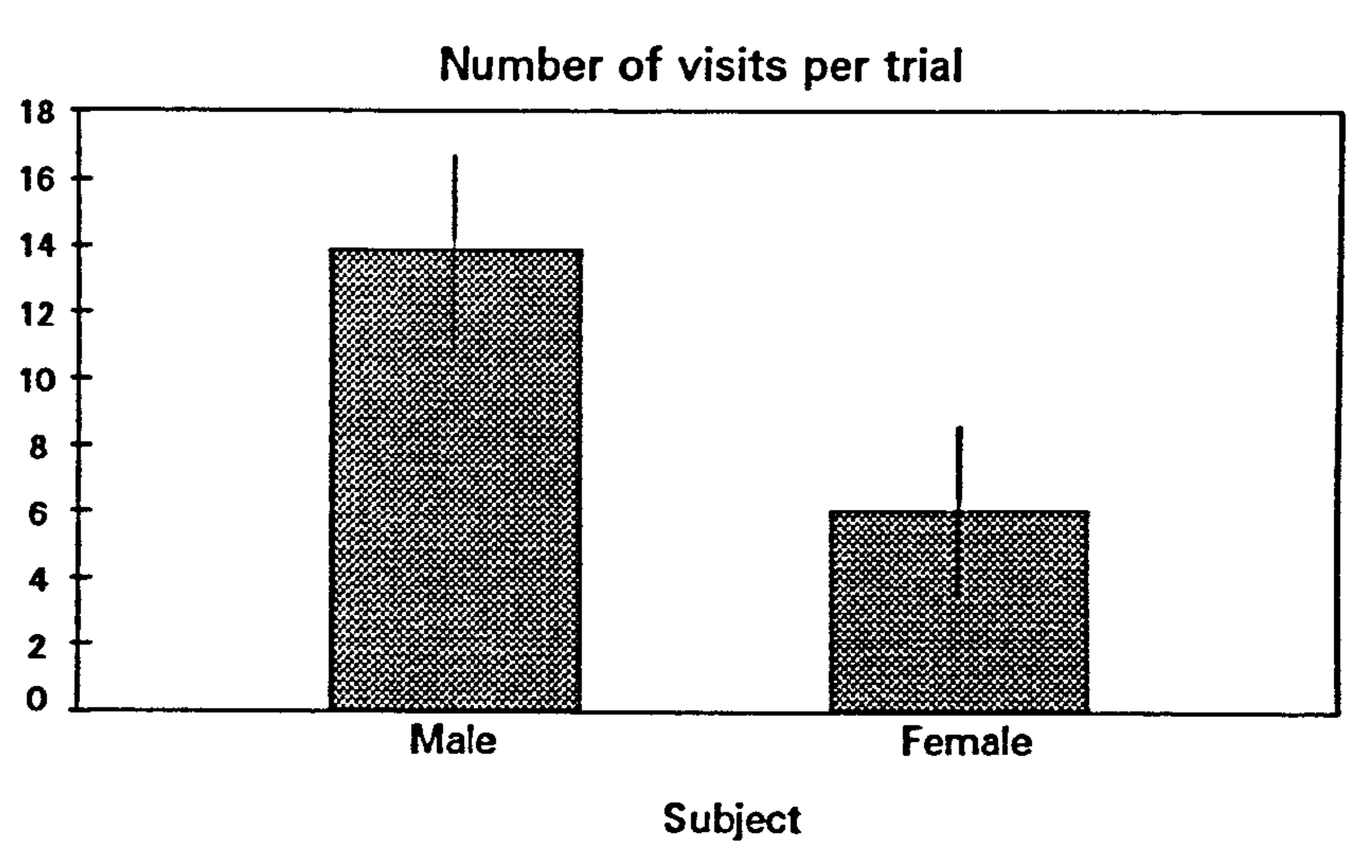
Plate 3.3. Faecal marking response to a tile marked by non-resident rats. The picture shows that the faecal marking response is very specific and localised around the tile, and that the number of faeces deposited was remarkably large. All the faeces were deposited overnight.

variable	Males			Females			Significance	
	mean	S.E	n	mean	S.E.	n	Z	p
Faeces deposited	10.4	6.21	5	0	0	4	3.24	0.001
Visits	13.85	2.93	5	6.08	2.55	4	2.43	0.01
Percentage of visits with faecal marking	20.05	9.95	5	0	0	4	3.24	0.001
Mean time on tile per visit (s)	64.36	20.56	5	15.85	1.22	4	2.75	0.01
Total time on tile per trial (s)	1082	502	5	100	35	4	2.55	0.01

Table 3.5. Difference (mean ± SE) between male and female response to urine marks deposited by non-resident rats.







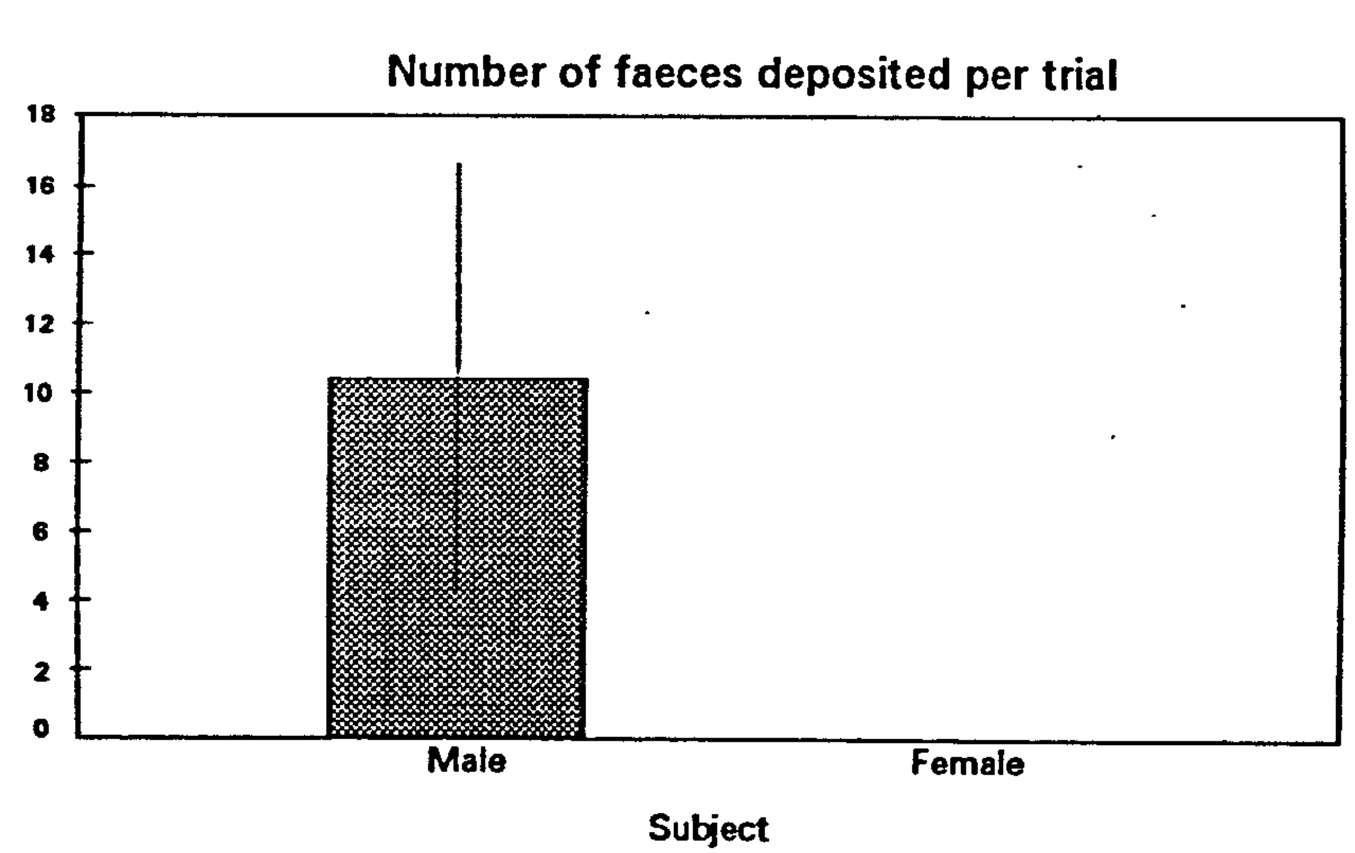


Fig. 3.6a. Response (mean ± SE) of wild rats to urine cues deposited by non-resident wild rats. The stimulus deposited on tiles is presumed to be a mixture of mainly male urine with small amounts of female urine. The variables shown are number of faeces deposited, number of visits, mean time per visit and total time per trial.

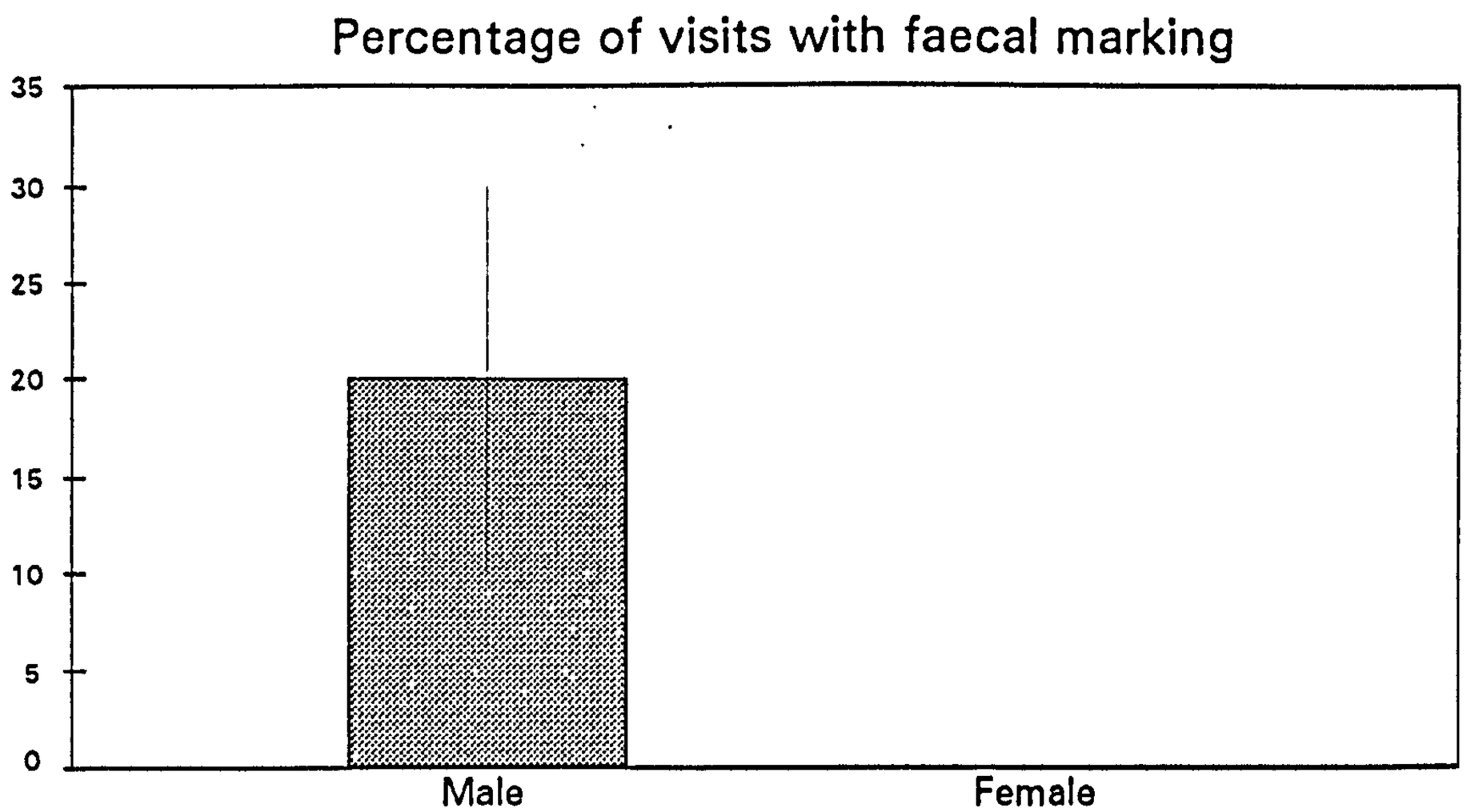


Fig 3.6b. Proportion (mean ± SE) of visits in which faecal marking was observed in response to urine cues deposited by wild rats from other colonies. The stimulus is presumed to be a mixture of mainly male urine with small amounts of female urine.

3.5.5. Discussion.

Rats faecal mark. The results strongly suggest that the defecation observed in response to urine stimuli is a type of scent marking behaviour involved in social communication. The deposition of faeces observed is unlikely to be due to incidental marking, i.e. it is unlikely that the difference in the amount of faeces found by each type of tile can be attributed to the difference in the time spent investigating each stimulus, for the following reasons:

i) In the pilot experiment (in which both tiles were video recorded) and in personal observations, rats were seen to stop at both tiles. Rats investigated the tile marked by rats from other colonies for longer than that marked by residents. The investigation lasted usually less than half a minute on both types of tile and

the difference between them was only a few seconds more when rats investigated urine cues from other colonies. In contrast, male rats stopped to groom themselves only after investigating alien scents. Faecal marking usually occurred during such grooming periods, which typically lasted around 5 minutes. Jolles, Rompa-Barendregt and Gispen (1979) have found that grooming is associated with high arousal states in laboratory rats. This suggests that rats stopped to groom by the tile with cues from other colonies because urine marks from unfamiliar or neighbour rats caused great stimulation, and also because it probably takes time to produce faeces with which to counter-mark. However this evidence is weak and based on the observer's impression.

ii) If faecal and urine deposition depended only on the time spent in a location, urine and faecal deposition should be correlated. That is, the ratio of urine marks deposited in response to non-resident versus resident stimuli should be roughly similar to the ratio of faeces deposited. However, although this ratio was 2:1 for urine marking (section 4.8), it was much greater for faecal marking (females showed no faecal marking).

iii) If faecal marking was incidental, males and females should faecal mark at similar rates, once corrected for differences in weight and metabolic rates. However, the results of table 3.5 show that the defecation rate was 10.4 ± 6.21 faeces/trial for males and 0 faeces/trial for females. Although the males visited and investigated the tile marked by rats from other colonies more than females did, female rats nevertheless stopped and investigated this tile, but they were not seen to deposit a single faecal pellet. The difference in faeces deposited is difficult to explain in terms of this difference in the number of visits because the visit frequency by females was half

that by males, whereas, as mentioned before, the males deposited at least 10 times more faeces than females. But faecal marking differences between males and females might still be explained as incidental marking because males spent 10 times more time in the area than females. However, the video tapes showed that the actual investigation times were similar between males and females, but only males spent additional time grooming themselves, and faecal marking occurred during this period (unfortunately the time spent by males investigating and grooming while in the monitored area was not recorded separately). The hypothesis that faecal deposition is incidental does not explain why faeces were seldom deposited (if at all) during the investigation time, and most of them were deposited after male rats had finished their investigation and started to groom themselves. If the rate of faecal production is similar for males and females, males might have had to spend much more time in the area monitored than females in order to *deliberately* deposit faeces intended for communication.

Faecal marking seems to play a role in social communication, since rats only faecal marked in response to urine stimuli from outsiders. If faecal marking was playing a role in self-orientation, and since olfactory stimuli only differed in the type of donor, a greater or similar rate of faecal marking would be expected towards resident compared to non-resident urine cues. In addition, if both males and females use faeces for orientation, it would be difficult to explain why females did not faecal mark at all.

The finding that male rats prefer to faecal mark in response to urine cues from non-resident rats suggests that faecal marking is not intended only for intra-group communication. However, it is not possible to conclude from this experiment that male faecal marking

only plays a role in communication between rat groups. If urine and faecal marking constitute status badges, male rats might be broadcasting a message to both rats within their own and those from other groups by clearly advertising their presence and status over any intruder who dares to urine mark their territory.

It might be argued that perhaps some of the urine deposited by residents may have dribbled under the tile when counter-marking, creating a strong stimulus for subsequent trials. Hence, rats would not be just responding to the alien urine marks, but also or perhaps mainly to the accumulated resident urine marks underneath and around the experimental tile. However, this does not seem very likely because the order of presentation and the location of the tiles were altered in four different locations per enclosure. Only in a few instances an unusually large amount of urine marking on the floor threatened to alter the faecal marking behaviour in subsequent trials. In those few occasions I faced a dilemma with two equally irregular outcomes: i) to clean the pool of urine and leave a clean area (looking similar to its everyday aspect although the odour profile would be altered) or, ii) leave the pool of urine (creating an unusually strong stimulus which may last for several days). The first option was carried out, tiles were removed in the morning and the area with pools of urine was washed and rinsed thoroughly. After the following trial, faecal marking was less scattered between the two tiles urine marked by resident (and laboratory rats, see next section), and faeces were found closer to each other and nearer the marks from laboratory rats. This showed that it was the urine cues from rats belonging to other colonies which produced the response and not a hypothetical building up of resident urine marks under the experimental tiles.

Faecal marking responses towards cues from neighbour and unfamiliar rats appeared to be similar. Fisher (see review in Temeles, 1994) hypothesised that the degree of aggressiveness with which an animal responds towards neighbours or unfamiliars depends not on its familiarity with them but with the degree of threat they pose: unfamiliars (wandering individuals without a territory also called floaters) may attempt to steal both mates and territory whereas neighbours are likely to steal mates but have their own territories (the threat they pose for other resources such as food or the enlargement of their territory at the expense of the resident's territory is likely to be of lesser importance). Unfamiliar individuals with territories are very unlikely to interact with a resident and do not pose a threat. Temeles (1994) found that a reduced aggressiveness towards neighbours (dear enemy phenomenon) only occurs in animals with either multi-purpose or breeding territories, and not in feeding territories where the threat of neighbours and unfamiliars is the same. Based on these findings and because wild rats have multi-purpose territories (section 1.4.5.1), they should show a reduced aggressiveness towards neighbours compared to unfamiliars. Such response appears to have been found in the experiment discussed in section 3.4, but not in that reported here. Brown (1992) found the opposite effect in the laboratory, greater investigation and marking by male resident rats towards familiar non-resident male urine marks compared to those of unfamiliar males, although the response observed may not be a territorial one because of the setting of his experiment. The similarity of response towards unfamiliar and neighbour male scents found in the present experiment might be due to the small size of the sample used (only five colonies) or the impossibility of controlling experimental

conditions in a specific way to test a difference in response towards neighbour and unfamiliar rats. Airflow might have made odour cues from any colony available to all the rest, regardless of the lack of olfactory communication at ground level between colony 8 and colonies 9 and 10. Alternatively, a rat may need physical contact with other rats to consider them as neighbours instead of as unfamiliar rats. Further test are needed to assess whether rats show a dear enemy phenomenon.

Sex bias in faecal marking. Although the unspecificity of the stimuli makes the interpretation of the sex bias in faecal marking inconclusive, the greater response of males is very suggestive. The urine marking response to clean tiles (section 4.3) showed that most of the urine marks deposited on clean tiles belong to the male. It seems likely that the individual who is faecal marking is responding to the most prominent odour cue in the tiles. Moreover, if, as indicated by Johnston, Chiang and Tung (1994), in golden hamsters scent marks deposited on top of previous marks mask them, it is likely that female urine would be masked by the more abundant male urine (although Johnston, Chiang and Tung (1994) did not tested the effect of sex in counter-marking). As a result males are likely to be faecal marking in response to male urine. If so, then faecal marking might be playing a role in inter-male competition (probably advertising their dominant status).

However, we cannot be sure of this conclusion because there was still a small proportion of urine from the female on the tile marked by the other colony. Males may be extremely sensitive to these urine marks and they may be responding to them. Alberts (1992) argued that animals compensate for the decreased volatility or

abundance of some scents by increasing olfactory sensitivity to them.

3.6. Faecal marking in response to urine cues from unfamiliar male and female rats.

3.6.1. Aims.

To determine whether individuals faecal mark differentially in response to urine cues deposited by rats of their own or the opposite sex, it was necessary to present urine stimuli deposited by males or females separately.

Scent marking in response to olfactory cues from individuals of one's own sex is likely to play a role in communication between competitors. That is because competition is more likely to be found between individuals of the same sex than between individuals of the opposite sex, as males tend to compete for mating opportunities (females being the limiting factor) and females tend to compete for nest sites or breeding opportunities (since male mammals are less likely to be involved in offspring care). Faecal marking only in response to individuals of the opposite sex, in contrast, is likely to be involved in sexual communication. However, these hypotheses are not mutually exclusive. Scents used for challenging possible competitors may attract mates, and, conversely, signals used to attract mates or display the qualities of the marking individual for mate assessment may also deter possible competitors.

3.6.3. Methods.

The methodology of this experiment was very similar to that of the previous one (section 3.5.3). The experiment consisted of a two choice test between previously clean tiles which had been

marked either by resident or unfamiliar rats. There were two treatments depending on the sex of the unfamiliar donor of the stimuli: male rats in one of the treatments and female rats in the other.

The experiment was conducted in three colonies housing pairs of rats (colonies 8, 9 and 10, see section 2.5) with five trials for each pen and treatment. The rats occupied the pens when these had been divided in half into 10 x 2.5 m enclosures. They were allowed one week after release for habituation to the enclosure before the set of experiments (including this one) began. This experiment was conducted at the end of the 4 week experimental period (i.e. one of the last experiments in the mentioned set).

Because of the limited availability of pens, and because of the great stress and disturbance that wild rats appeared to suffer when they were kept in cages, caged laboratory rats were used as donors. Unlike wild rats, laboratory rats did not avoid the tiles introduced in their cages. Instead, they marked them profusely, presumably as any unmarked part of their small home range. Thus, the tiles probably became strong stimuli for the wild rats. Three male and three female Wistar laboratory rats were used as donors (see table 3.6 for their weights). All the laboratory rats were kept in cages (described in section 2.6) in the same room (in the CSL animalarium, a building separated from the rat shed) under isolation conditions. Water and food were supplied ad libitum. Due to reasons explained in section 2.6, subadult individuals (six weeks old when the experiment started) were supplied instead of adults. Therefore, a sexual response towards their urine was less likely than if adult rats had been used as originally designed.

Table 3.6. Weights of laboratory rats that marked clean tiles used as experimental tiles.

Identification number	Male	Female
1	326	209
2	322	220
3	351	213

Collection of stimuli. A clean tile was exchanged with a familiar tile of each pen. Wild rats marked it overnight and it was then used as a control (bearing urine marks from residents). Another clean tile was introduced in one of the cages housing a laboratory rat. Again the rat marked it overnight and it was used as an experimental tile (bearing marks from an unfamiliar rat).

Behaviour recorded. On the night the trial started, both tiles were sited consecutively occupying the position of a pair of familiar marked tiles by a wall (and therefore, as explained in section 2.6 placed 1 m apart). Faecal marking was recorded the following morning as the number of faeces found within 0.5 on either side of each tile (section 3.5.3).

As in the previous experiment, behaviour of each sex was recorded at the experimental tile. The variables measured were the same as in the previous experiment. Records were taken on video for 11 h (1800 to 0500 h) with the equipment described in sections 3.5.3 and 2.6. Thus, trials started before rats typically were active.

Due to restrictions in the number of sites where the camera could be mounted or pointed towards, only two different locations for each tile were used (four in total, see section 3.5.3). The location of experimental and control tiles was alternated between these two locations to reduce the possibility that the urine or faecal marks left

cues that rats responded to subsequently instead of responding to the stimuli on the tiles, and also to avoid rats getting used to find the same stimuli in the same place (or responding more strongly to one stimulus because of its location).

To balance the presentation of stimuli, laboratory rats marked the tiles for different pens in sequence order (e.g. male laboratory rat no. 1 would mark a tile for colony 8 on day 1, for colony 9 on day 2, for colony 10 on day 3, for colony 8 on day 4 and for colony 9 on day 5). To avoid habituation to one type of stimulus, rats were presented with male and female experimental stimuli on alternate days (first day from males, second day from females, etc.). The order in which the rats encountered the stimuli tiles when coming out from the nest was also alternated (e.g. for tests using male stimuli, on trial 1 the experimental tile was closer to the nest, on trial two, the control tile was closer, etc.), for reasons explained in section 3.5.3.

Statistical analysis. Two sets of analyses were conducted. One examined the amount of faeces found at the end of each trial in response to each type of urine stimulus. The other group of analyses involved male and female behaviour recorded at the experimental tile. Because the analysis required tests of specific hypotheses, and data did not appear to be normally distributed, non-parametric tests were used.

-Tests of the number of faeces deposited near tiles. A general-test two-way non-parametric ANOVA tested the effects of the sex of the unfamiliar urine donor and trial order on the difference between the number of faeces found by the experimental and control tiles. Faeces found by unfamiliar male marks were subtracted from faeces found by the matching control tile and

compared to the difference in faecal counts when the experimental urine marks belonged to an unfamiliar female. Data were ranked within pens to take into account the fact that the response of the rats on different days was not independent. Because the response included both that of the male and the female, no difference was predicted in response to male urine compared to female urine. This was because, although males were expected to respond more strongly to male than to female marks, there was a possibility that females might show a competitive faecal marking response towards female marks when these were presented separately, counteracting the expected decrease in faeces produced by the male towards female urine marks (see previous section).

A specific-design two-way non-parametric ANOVA tested whether there were more faeces by the experimental tile (bearing unfamiliar marks) than by the tile marked by residents and whether trial order had any effect. If faecal marking response to unfamiliar marks was similar regardless of the sex of the donor, data from both responses would be pooled, otherwise a separate ANOVA would be required for each sex. Data were ranked within pens to take non-independence of the data into account. As in the previous test, the response to unfamiliar marks was expected to be greater than towards marks from resident rats in every trial. Thus the coefficients for a test were:

	Trial (1-5)				
Unfamiliar male/female	+1	+1	+1	+1	+1
Resident	-1	-1	-1	-1	-1

-**Residents' behaviour at the experimental tile.** A series of general-test two-way non-parametric ANOVAs examined the effect of day on male and female towards marks from unfamiliar individuals (the response to male and female marks was pooled for this analysis). Because both treatments alternated on subsequent days and the faecal marking response seemed quite strong, no significant day effect was expected. Subsequently, the mean response per trial was examined with a specific-test two-way non-parametric ANOVA to test whether residents showed a greater response towards urine from unfamiliar individuals of their own sex. The data were ranked within each pen in both cases to compare male and female across days in the first test, and to compare their response to marks from unfamiliar males and females in the second. The coefficients for the specific test were:

	Male urine cues	Female urine cues
Male response	$\lambda = +1$	$\lambda = -1$
Female response	$\lambda = -1$	$\lambda = +1$

The variables measured were those recorded in the previous experiment (section 3.5.3).

3.6.4. Results.

-**Faeces found by tiles.** Wild rats showed a similar preference to faecal mark the unfamiliar urine cues regardless of the sex of the donor (H_{1df} = 0.00, ns). There was no habituation effect (H_{4df} = 1.93, ns) nor any interaction between sex of the donor and trial

(H_{4df} = 0.61, ns). The number of faeces found near each type of tile in response to male and female urine marks was thus pooled to test whether the response to unfamiliar cues was greater than that towards from own colony urine. As predicted, rats faecal marked more in response to unfamiliar urine marks compared to those of residents (Z = 5.07, $p<0.001$; mean number of faeces by unfamiliar marks ± S.E. = 9.2 ± 5.2 n=3, faeces by marks from residents ± S.E. = 1.3 ± 1.0 n=3; see fig. 3.7).

-**Behaviour at the experimental tile.** Males responded more strongly than females. When the overall response to unfamiliar urine cues was considered (i.e., pooling data of responses to male and female urine) males showed a consistently greater response than females with respect to all of the variables considered concerning both their faecal marking and investigation (mean time, number of faeces deposited, etc., see table 3.7). There were no day effects (faeces deposited, H_{4df} = 0.81, ns; mean investigation time per visit, H_{4df} = 1.16, ns; total investigation time per trial, H_{4df} = 0.70, ns; number of visits per trial, H_{4df} = 1.77, ns; number of marking visits per trial, H_{4df} = 1.24), nor any interaction between sex of the resident and day (faeces deposited, H_{4df} = 0.80, ns; mean investigation time per visit, H_{4df} = 2.22, ns; total investigation time per trial, H_{4df} = 3.40, ns; number of visits per trial, H_{4df} = 3.69, ns; number of marking visits per trial, H_{4df} = 1.13).

Because no day effects nor interaction with resident's sex were found, the mean response per trial was examined to test the effects of the gender of resident and that of the unfamiliar donor of the marks. As predicted, individuals showed a stronger response towards urine cues from their own sex than towards those from the

opposite sex (table, 3.7 and figure 3.8a and 3.8b). Females were only seen faecal marking in response to female urine cues, not in response

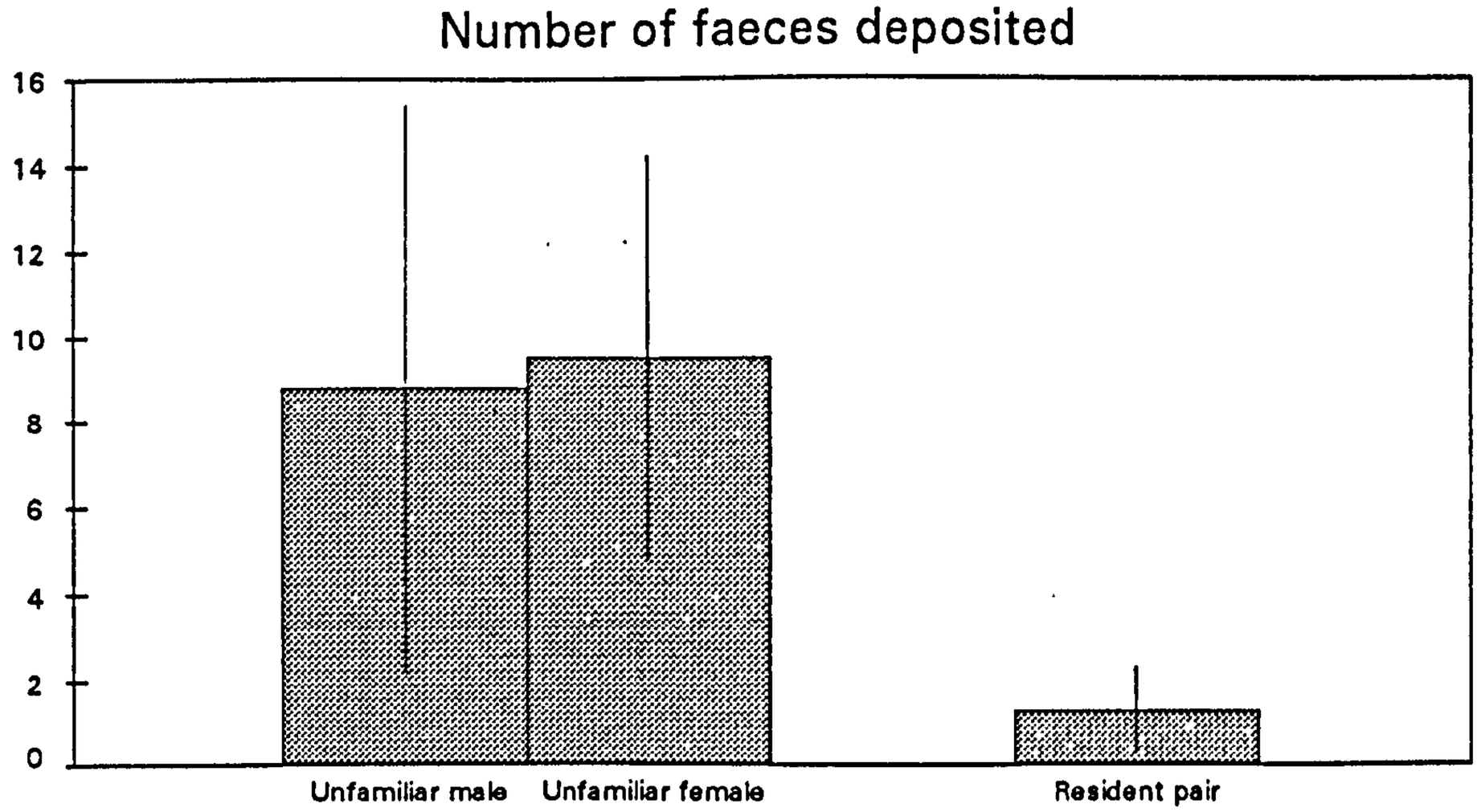


Fig. 3.7 Number of faeces (mean ± standard error) deposited by wild rats in response to urine marks deposited by either laboratory rats (male and female presented in different trials) or resident rats.

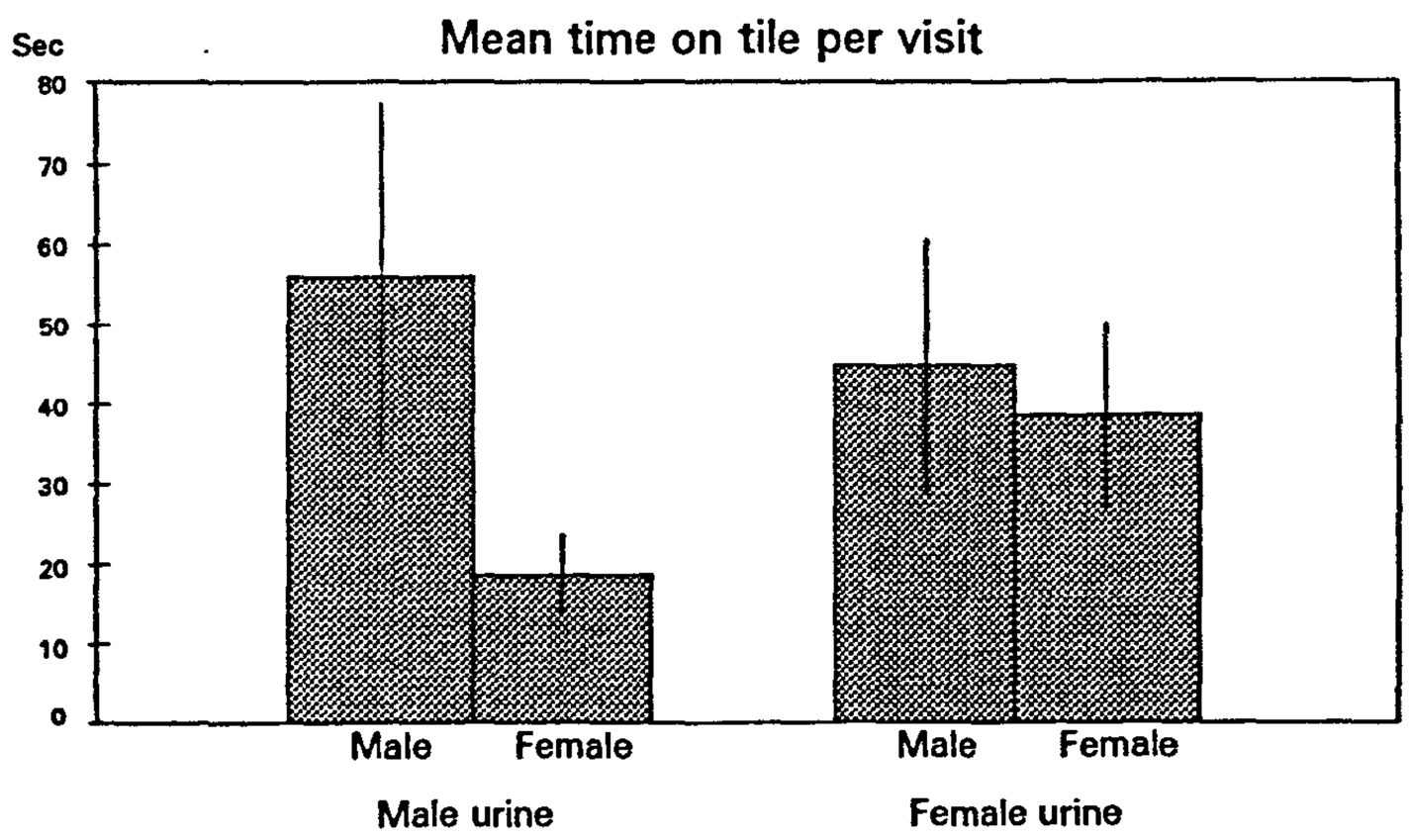
to male urine cues. Individuals responded significantly more to cues of their own sex in respect to the following variables: faeces deposited per trial, mean time on tile per visit, total time on tile per trial and proportion of visits in which faecal marking occurred. The tendency failed to reach statistical significance for the number of visits per trial. The results showed that male response was only slightly weaker towards female than towards male marks. Females, however, showed a strong response towards marks from other females, similar in strength to the response displayed by males, and a markedly weaker response to male marks.

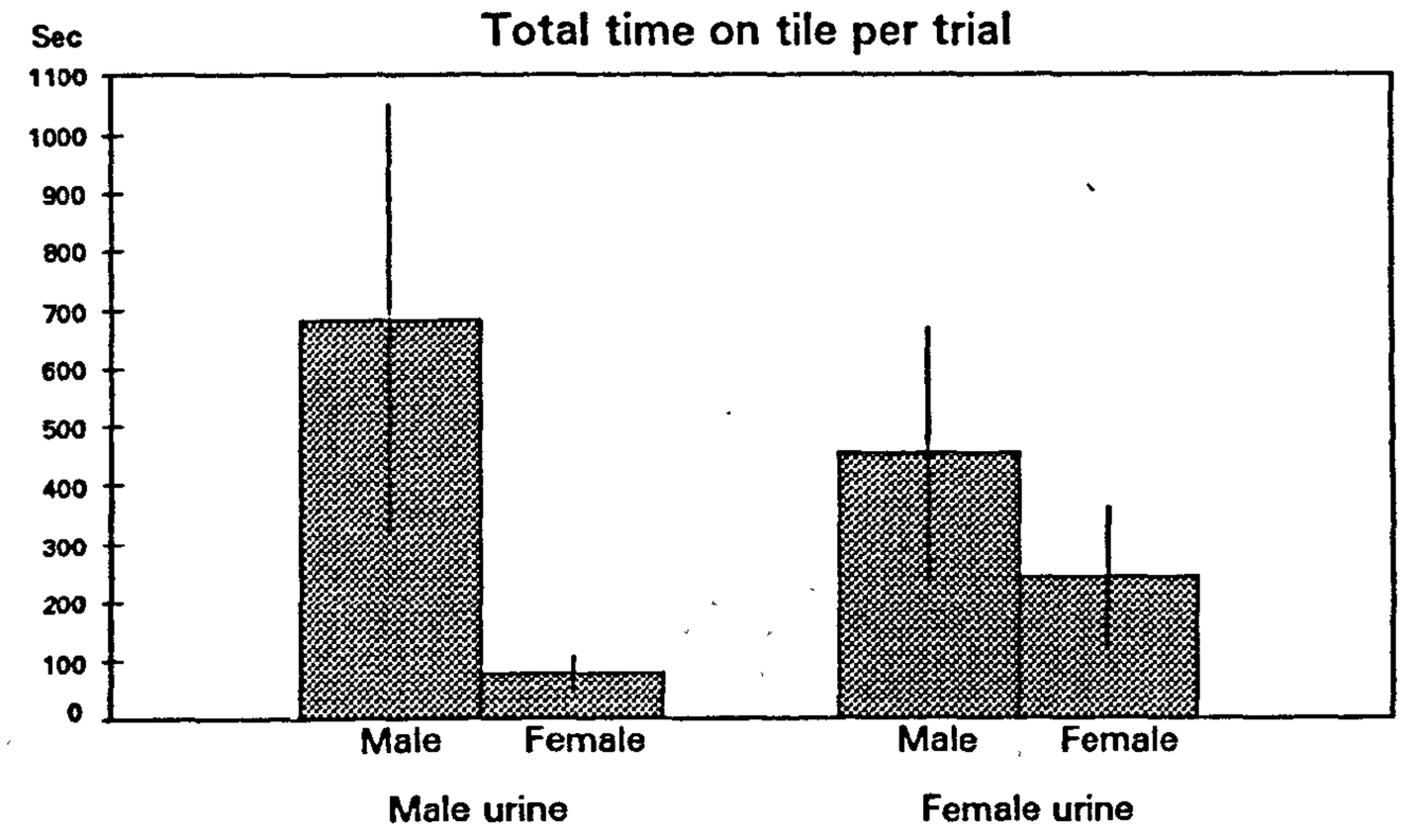
Table 3.7. Comparison of male and female response (mean ± S.E.) to unfamiliar urine cues (response to urine from males and females pooled). F, faeces deposited; MT, mean time on tile per visit; TT, total time on tile per trial; VIS, number of visits per trial; MKVIS, proportion of visits in which faecal marking occurred.

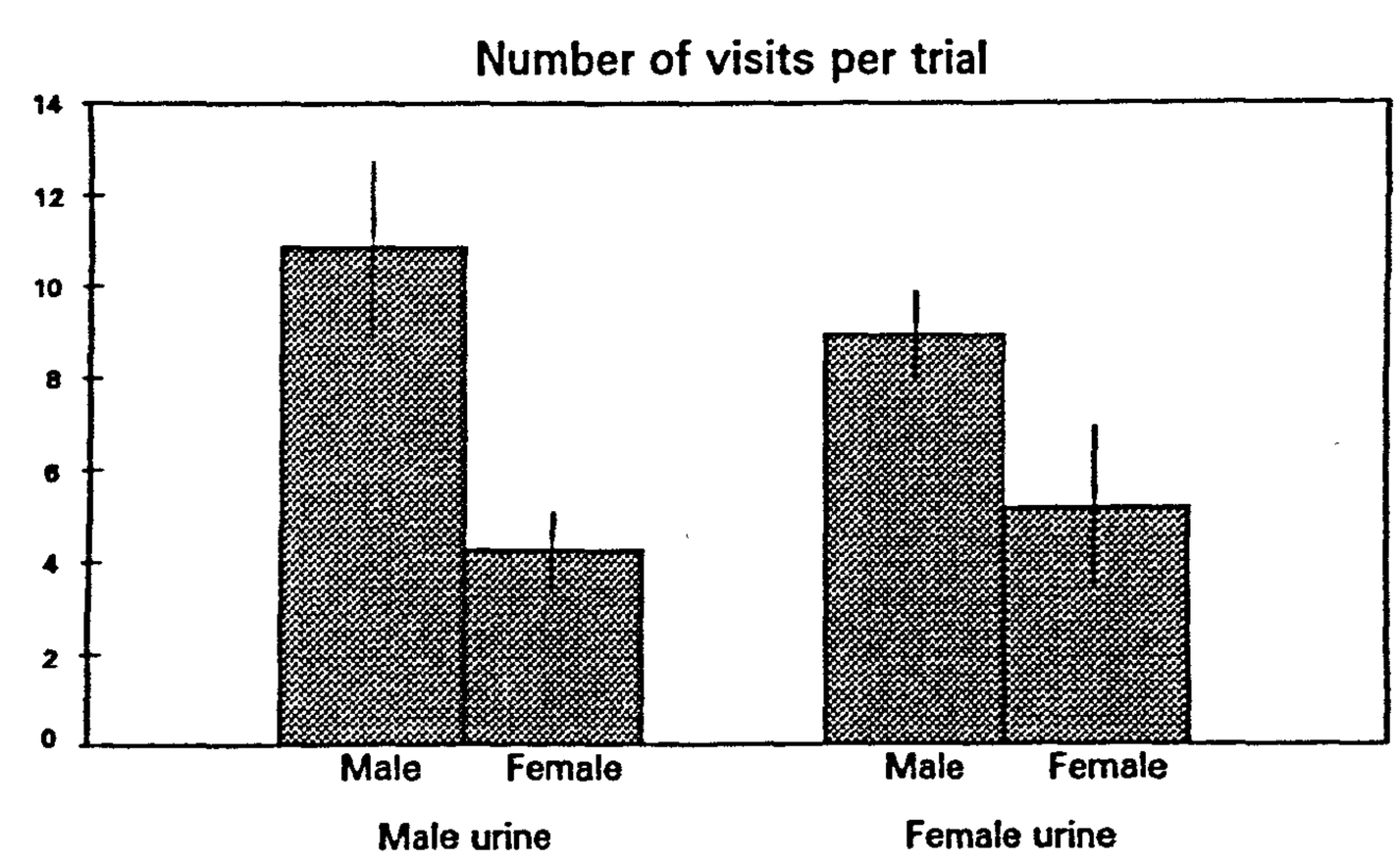
	Male response			Female response			Significance	
Variable	Mean	S.E.	n	Mean	S.E.	n	H_{1df}	p
F	5.63	4.24	3	1.01	0.57	3	12.12	0.001
MT (s)	50	19	3	29	14	3	13.58	0.001
TT (s)	569	289	3	168	77	3	23.00	0.001
VIS	9.93	1.27	3	4.77	1.33	3	33.09	0.001
MKVIS (%)	16.26	11.71	3	6.66	2.33	3	11.83	0.001

Table 3.8. Comparison of the response (mean ± S.E.) of male and female wild rats to urine from unfamiliar male and female laboratory rats. M, male; F, female; FC, faeces deposited; other acronyms as in table 3.7.

	Sex of	Male response			Female response			Significance	
Variable	donor	Mean	S.E.	n	Mean	S.E.	n	Z	p
FC	M	6.87	5.31	3	0	0	3	1.78	0.05
	F	4.40	3.19	3	1.87	1.01	3		
MT (s)	M	56	22	3	19	5	3	3.04	0.01
	F	45	28	3	39	12	3		
TT (s)	M	684	366	3	79	26	3	2.80	0.01
	F	454	216	3	243	117	3		
VIS	M	10.87	1.91	3	4.28	0.81	3	ns	
	F	9.00	0.92	3	5.20	1.74	3		
MKVIS (%)	M	17.33	13.41	3	0	0	3	1.64	0.05
	F	15.38	9.71	3	11.55	3.09	3		







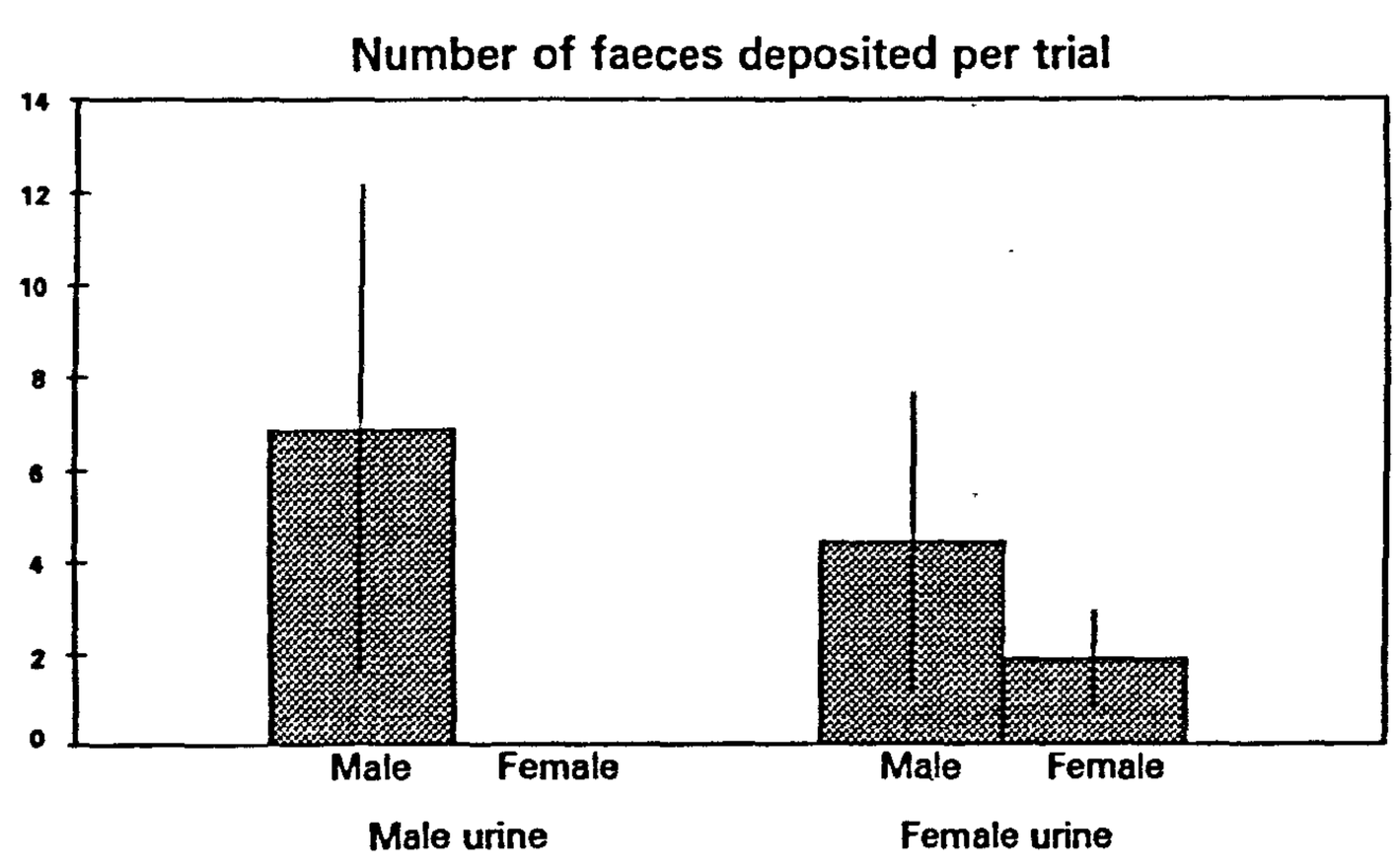


Fig. 3.8a Response of wild rats (mean ± SE) towards urine cues deposited by either male or female laboratory rats. The variables shown are number of faeces deposited, number of visits, mean time on tile per visit and total time on tile per trial.

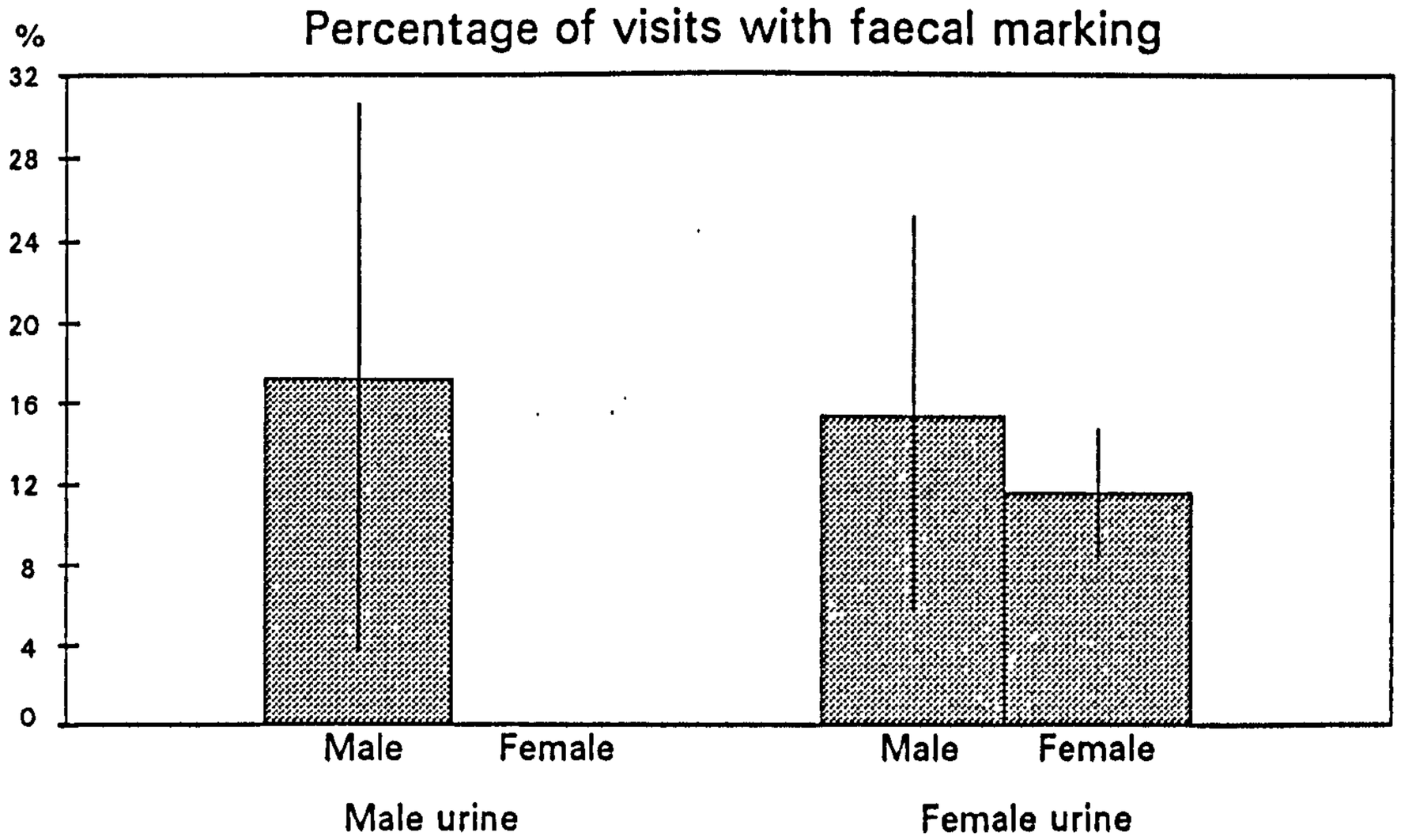


Fig 3.8b. Response of wild rats (mean ± SE) towards urine cues deposited by either male or female laboratory rats. The variable shown is the proportion of visits in which the individuals faecal marked.

3.6.5. Discussion.

The results of this experiment corroborate my finding that rats faecal marked in response to certain urine cues (section 3.5). Faecal marking cannot be explained as incidental marking because not only is it aimed at urine marks from intruders, but it tends to be sex specific, i.e. marking was greatest when olfactory cues belonging to individuals of the same sex as the marking rat were present. Figure 3.8a shows that, although the total amount of time per trial that female rats spent investigating male and female urine cues was similar, they only faecal marked in response to female urine and never in response to male urine.

Faecal marking appears to be used to advertise the presence of the resident rats. This seems to be aimed at potential competitors, although a number of additional hypotheses would also explain the results obtained. The line of evidence suggesting the role of faecal

marking in competitive behaviour is the following: as in the previous experiment, faecal marking was aimed mainly towards urine cues from rats belonging to other colonies. Furthermore, males investigated and marked more than females did. This is consistent with findings of R. J. Blanchard et al. (1984) who observed that the dominant male was responsible for most of the attacks towards intruders (60-80%). Also, wild males investigated and faecal marked urine marks from unfamiliar males and females similarly, although the response towards males tended to be slightly stronger. This may suggest that males regard rats of both sexes from other colonies as potential competitors, as mice do (Hurst, 1990c). Because singly housed individuals, at least in mice, show many physiological and behavioural similarities to territory owners (Brain, 1975), resident rats may have regarded territorial marks from immature individuals (even those from females) as a great challenge by individuals of poor fighting abilities. Observations from wild and laboratory rats showed that males attack females, although the response is infrequent and less aggressive than towards males (Barnett, 1958; Calhoun, 1962; Alberts and Galef, 1973; Thor and Flannelly, 1976b; Barnett, Dickson and Hocking, 1979). However, other alternatives are possible because scent marking may play more than one role at the same time. Thus, it is possible that the male response towards urine from unfamiliar males constitutes a competitive response whereas his response to marks from unfamiliar females is sexually motivated (although, as these were immature, this hypothesis is very unlikely). In addition to its apparent role in inter-male competition, the male response to males may be aimed at attracting possible mates by showing intolerance towards intruders.

Finally, the stronger marking response towards urine from the rat's own sex (particularly in the case of the females) also suggests that faecal marking plays a role in warning potential competitors. That is because animals tend to compete with individuals of their own sex more than with those of the other (e.g., males tend to compete for mating opportunities and females tend to compete for nest sites or breeding opportunities). Nevertheless, intersexual competition could occur (mainly for food). However, in the experimental set up discussed here, competition for food and therefore, intersexual competition was unlikely because there was food ad libitum distributed in more than one site.

Because the female wild rats in the pens were usually pregnant, their strong response towards urine from alien females might be a means to defend their breeding status, as mice appear to do (Hurst, 1990b), or an intent to prevent immature intruder females from attempting to settle and compete for a breeding opportunity in the future. Zyporin and McClintock (1991) found that the dominant female is the first to become pregnant. Although some researchers have found that all colony members contribute to defend the territory (Blanchard et al., 1975; Adams and Boice, 1989), females should not advertise their presence to males because they are unlikely to win should a fight arise, especially if the intruder male is bigger (Flannelly and Flannelly, 1985). In this case however, male donors were presumably of poor fighting ability because of their young age, although the effect of isolation may have produced similarities in their urine composition or distribution on tiles similar to those of territory owners (see above).

Not all researchers have found a greater aggressive response towards females than towards males. Albert et al. (1988) found that

lactating female laboratory rats attacked intact males more than females. However, this finding does not contradict the results presented here. The rats in Albert's paper were forced to live with an unfamiliar male or female continuously after giving birth. In such a situation, females are likely to attack a male more than a female as the former may attempt infanticide of her offspring to accelerate her oestrus (Brown, 1986a). For intruders trying to get established in the resident territory, females are more likely to fight against females not only because they are less likely to win against a male, but also because the resident male should be more likely to accept another female than another male. In addition, in colonies of rats, the dominant male is already defending the territory against male intruders (Blanchard, R.J. et al., 1984) with better chances of success than resident females have.

As mentioned before, other hypotheses could possibly explain these results, although they may not exclude the previous ones. For example, females may reduce their rate of marking in response to males in order to avoid attracting them if they are not ready to mate. To discern between these possibilities, experiments are needed examining the behaviour of both male and female resident rats towards adult male and female non-resident rats.

CHAPTER FOUR
URINE MARKING.

4.1-Introduction.

Urine marking is the most studied type of scent marking behaviour in rats. Mammal urine is eliminated frequently and in abundance, and in its composition contains information regarding the internal state of the donor (Albone, 1984). It is thus not surprising that many mammals use it for chemical communication.

Both laboratory (Boice, 1977) and wild rats (Calhoun, 1962; Telle, 1966) mark their pathways with urine. These pathways are usually sited close to cover or to vertical surfaces like walls (Calhoun, 1962; Telle, 1966; Taylor, 1978). In the laboratory, urine is found at higher concentration around the periphery than in the centre of a rat's cage (Peden and Timberlake, 1990). Urine marks are also deposited on objects (Brown, 1975; Price, 1975; Hopp and Timberlake, 1983), and at burrow entrances (Hopp and Timberlake, 1983).

Urine marking may play a role in orientation. Rats mark some areas of their home range, like paths and burrows, more frequently than others. They move at high speed along these paths or runs (Recht, 1982), but move only slowly, constantly sniffing the substratum, outside them (Telle, 1966). Telle (1966) also found that when wild rats were introduced to an area previously occupied by other rats, they started to move along the existing urine trails. This suggests that urine marks on these runs might indicate that these are obstacle free or safe pathways. Galef and Buckley (1996) have recently added another possible role that trails (presumed urine trails) left by rats may play in orientation: they may provide

information about feeding sites. Galef and Buckley (1996) found that trails left by recently fed rats attracted conspecifics, but not those left by rats who did not feed. They also found that increasing the number of individuals producing the trail increased its attractiveness, but this effect did not depend on the amount of food eaten per rat. In addition, rats could not detect the direction of the trail. Although these trails appear to correspond to the urine trails observed by Calhoun (1962), Telle (1966), and Boice (1977), Galef and Buckley (1996) did not test whether urine was responsible for the attractive effect, nor that it was present in the trail.

Differences in the rate of urine marking between males and females may support the hypothesis that urine plays a role in social communication (sexual or competitive advertisement). For example, because the dominant male rat is the individual most involved in attacking intruders (Blanchard, R. J. et al., 1984b), a greater rate of urine marking by male rats might suggest that urine plays a role in competitive advertisement. In contrast, an increased urine marking rate at oestrus by female rats would suggest that urine is involved in sexual communication (mate attraction). However, these differences do not demonstrate the role scents play conclusively: a greater urine marking rate by males may also constitute a sexual display (mate assessment/mate attraction), or it might be a by product of the larger size of males with no role in communication. Furthermore, both competitive and sexual advertisement hypotheses are not mutually exclusive and may be working at the same time.

Urine seems to play a role in rat sexual communication. Female wild (Calhoun, 1962) and laboratory rats (Birke, 1978; Birke and Sadler, 1984; Lee, Mitchell and Adams, 1984; Matochik, White and Barfield, 1992; but see Peden and Timberlake, 1990) urine mark

more frequently at oestrus or pro-oestrus. Male wild rats respond to marks from receptive females by investigating and counter-marking them with urine (Calhoun, 1962). A similar response for investigation was found in laboratory rats (Carr, Loeb and Dissinger, 1965; Carr, Wylie and Loeb, 1970; Brown, 1977), but apparently not with respect to urine marking (Brown, 1977, 1991; Birke and Sadler, 1984).

The literature appears to be contradictory with regard to sexual differences in the rate of urine marking: some researchers have found that males investigate and urine mark more than females do (Price, 1977; Brown, 1991); other researchers have found that females mark more than males (Lee, Mitchell and Adams, 1984; on the burrow entrance: Peden and Timberlake, 1990); and yet others have found no difference in urine marking between the sexes (Birke and Sadler, 1984; on objects: Peden and Timberlake, 1990). However, this research may not be representative of wild rats in a natural setting because it has been carried out in laboratory cages with domestic strains, and this may have influenced both the social hierarchy of the rats, and their scent marking behaviour (section 1.4.3).

Difference in time spent that rats show when investigating urine marks may also suggest the role that urine plays in social communication. A greater time spent investigating urine from individuals of the subject's own sex might indicate that urine is involved in communication between competitors. In contrast, urine might play a role in sexual communication if rats investigate urine from rats of the opposite sex more than urine from their own sex. Most studies report greater investigation and marking towards marks made by members of the opposite sex. Thus, dioestrous

female rats mark male urine more than female urine (Birke and Sadler, 1984). Females investigate urine marks from both intact and castrated male rats more than marks from either intact or castrated females (Brown, 1977, 1991), although they urine mark urine cues from males and females (intact or castrated) at a similar rate. Males investigate and mark female more than male urine (Brown, 1977, 1985c, 1986c, 1991; marking alone: Brown, 1992). They also investigate female more than male conspecifics (Flannelly and Blanchard, 1982). Gao (1991) found that rats did not prefer to stay in an area with urine of their own sex more than in a clean area constituting the control, but they preferred an area with urine of the opposite sex more than the control. These results should, however, be considered with great caution, because the experiments were conducted with laboratory rats isolated from contact with members of the opposite sex (and usually reared in same sex groups) and in clean unfamiliar laboratory cages (section 1.4.7.2.5).

Rats also seem to use urine in communication between competitors. Intruders urine mark when placed inside an already occupied laboratory cage (Adams, 1976). Residents respond both by urine marking the alien marks and by attacking the intruder. After being defeated by the resident the urine marking rate of the intruder drops. Further evidence supporting the involvement of urine in communication between competitors is that the rate of urine marking among males seems to be related to their level of aggression. Aggressive males urine mark more than less aggressive males (Taylor, Bartko and Farr, 1987).

In summary, rats seems to produce an uneven pattern of urine marking, concentrating urine in trails that seem to play a role at least in orientation. Evidence showing that females increase scent

marking (urine and flank marking) at oestrus suggests that urine marking plays a role in mate attraction. The fact that urine marking precedes aggression and that the rate of urine marking decreases in the defeated individual suggests urine marking also plays a role in communication among competitors. A preference to investigate and mark in response to scents from their own or the opposite sex might suggest the likely roles that urine plays in communication. Most publications report a greater investigation and marking of scents from the opposite sex, suggesting a role in sexual communication, but the experiments seem to have been designed specifically for testing roles in sexual communication. The evidence on differences in marking rate between males and females is also controversial, although if this was assessed carefully, this might provide help in understanding the role that urine plays in communication.

The general aims of this line of research were: i) to corroborate the existence of such an uneven distribution pattern of urine under controlled conditions, and assess whether this occurred due to incidental deposition or not; ii) to assess the role of urine marks in orientation and social behaviour by measuring the rate of urine marking by males and females, and their responses to manipulations of the olfactory background, with respect to both social (introducing marks from unfamiliar individuals) and non-social (introducing clean objects) stimuli.

4.2. Pattern of urine marks.

4.2.1. Aims.

The density of urine marks was assessed in different areas of the rat's enclosure (section 2.3) because this information may suggest possible roles for urine marking in orientation and social communication (e.g., if a greater density of urine marks was found around food resources this might suggest that urine cues could play a role in signalling feeding points). In addition, understanding the natural pattern of urine marks was an important prerequisite to assess the most appropriate areas for conducting experiments involving the manipulation of urine marks.

4.2.1. Preliminary observations and pilot study on urine marking.

Preliminary observations. The aim was to observe the urine marking behaviour of wild rats, how they used their home range and to corroborate the descriptions of urine trails found in the literature.

As explained in section 3.2.1, a large colony of wild rats (colony 1) was observed in their home enclosure in order to assess their scent marking behaviour. The colony was formed by 17 rats and was observed in the enclosure they had occupied continuously for 7 months. The observation period lasted four weeks and took place before they were transferred to an adjacent pen for testing. The observations were conducted for five hours a day (from 2000 to 0100 h) which included the first peak of the daily activity of rats (Calhoun, 1962; Barnett, 1975; Nieder 1985; and personal observation). The enclosure was carefully examined for cues of urine marks and their location.

Individuals generally walked next to the enclosure walls, avoiding open areas. They usually moved slowly and cautiously, sniffing the substratum when in the centre of the pen. In contrast, they moved faster and less cautiously along the walls. The darkened white colour of the floor paint by the walls suggested that the density of urine marks was greater in these areas than elsewhere. My observations seemed to be consistent with those from the literature on urine trails, but there was no proof that the darkened trails by the walls corresponded to the urine trails described by Calhoun (1962) and Telle (1966). There did not seem to be an obvious increase in urine density around food bowls, as Laland and Plotkin (1991) found in laboratory rats, but this observation should be taken with great caution because it was not validated with tests. The area around the water pot seemed also darkened. However, this was likely to be due to water spillage attracting dust, because the dusted areas irradiated from the very edge of the pot, and the genitals of the rats were never seen close to the edge of the pot.

Pilot study. The aim was to examine in greater detail the build up of the pattern of urine marks suggested by the observations above before investing in a costly study replicated over several colonies.

This pilot experiment was conducted in the pen to which the large colony was transferred for testing. Urine marking on the substratum was sampled using quarry tiles as described in section 2.6, using a 0.5 cm^2 grid to measure coverage. The enclosure had 23 quarry tiles, each with a metal rod attached to its centre, which were placed in several areas of the enclosure to compare the density of urine marks. Fifteen of these were located in the centre of the pen, four tiles were placed by the walls used by the rats in their trips

from the nest to the feeders, and one tile was located at each corner of the enclosure. Measures were taken daily for 30 days. Tiles were examined in the morning, when rats were not active.

Some pilot experiments conducted simultaneously with this study involved the exchange for one hour (between 2100 and 0100 h) of tiles between the centre and those by a wall. This did not seem to disturb the pattern of the marks deposited on the tiles. However, one of the tiles at the centre was mistakenly left by the wall overnight instead of replacing it back at its original position after the end of the 1 hour trial. Thus, it was discarded from the records. Rats urine marked it abundantly, probably responding to it as towards other tiles belonging to the walls.

The rods, instead of promoting urine marking (as described by Hopp and Timberlake, 1983, and Peden and Timberlake, 1990) might have deterred the rats because urine marking on the tiles was very scarce, whereas all later colonies marked tiles without rods abundantly. The urine marks found were distributed as follows:

-Urine marking on the tiles in the centre (which occupied an area about 2 x 3 m with its longer axis parallel to that of the hay stack) was almost non-existent (mean percentage ± SE = 0.02 ± 0.04 %, n=14).

-In contrast, all four tiles placed by the wall were urine marked to some extent (mean percentage ± SE = 1.21 ± 0.08 %, n=4).

-The tiles at the corners had a greater coverage than the tiles by the wall (mean percentage ± SE = 3.13 ± 1.69 %, n=4), especially those in the corners near the hay stack (mean percentage ± SE = 5.93 ± 1.33 %, n=2), which were visited more frequently.

The pattern of marks appeared to agree with that found in the previous enclosure.

4.2.3. Distribution of urine marks in open areas.

4.2.2.1. Aims.

The aim was to conduct a formal study to corroborate the differences found between the density of urine marks in different areas of an enclosure, and to assess the stability of the pattern during the period when rats were studied (about 4 weeks per colony).

4.2.3.2. Methods.

Measurements were conducted in the enclosures housing colonies 4, 5, 6 and 7 (section 2.5). Each pen housed a pair of adult wild rats. The rats had been caught in the wild by the staff of the Central Science Laboratory. They were transferred to small individual cages for two days to collect faeces and urine for experiments reported in sections 3.4, and 4.7. As mentioned in section 2.5 and chapter 3, a female was found dead in one of the pens (colony 5). Despite that, data from the remaining male were included in the analysis. It was impossible to determine the cause of death. No other casualties occurred in any pen.

To eliminate any possible scent cues, all the enclosures were swept, vacuum cleaned, washed with water and liquid detergent, rinsed and left to dry for at least two days before releasing the rats. A fresh stack of hay was introduced before releasing the rats.

Urine deposition was sampled in all open areas of the enclosure, using one tile per square metre of open floor (section 2.6). Thirty eight quarry tiles were used. Twenty six of the 38 tiles were

placed by the walls, and 12 in the central area. The area occupied by the walls corresponded to 26 m^2, whilst the central area consisted of the rest of the enclosure except that occupied by the hay stack, feeding bowls and water pot (12 m^2). Tiles placed in corners of the enclosure closest to the hay stack were not measured because the experiment reported in section 3.4 was carried out using these and was likely to affect urine marking. Thus, the area occupied by the wall tiles monitored in the study was 24 m^2. These were sited in contact with the wall while the rest were placed at the centre of each square metre. Rods were not attached to any of the tiles because these appeared to deter urine marking in the pilot experiment. In colonies 6 and 7 an extra tile was laid at both sides of the pipe that allowed olfactory communication between pens (section 2.3). The aim was to compare urine marks deposited in areas of incoming neighbour odours with those deposited in adjacent areas.

The tiles used were either new or reused, but in both cases they were thoroughly scrubbed with liquid detergent, rinsed and then left to dry for at least one day.

Urine marks were recorded in the morning every two days using a 0.75 cm^2 grid as described in chapter 2. However, when most of the surface of the tile was covered by urine marks, the number of *unmarked* squares was counted and then subtracted from the total number of squares (784) to compute the area marked. Urine spots were recognisable as dark spots with a glassy surface. Because the rate of urine marking in colonies 6 and 7 was much greater than in colonies 4 and 5 and because the amount of work for a single record became progressively unbearable (records reached 20,000 squares per pen in the last counts), the build up of urine marks was monitored for 30 days in colonies 4 and 5 but only for 18

days in colonies 6 and 7. For statistical analysis, data were compared for the first 18 days only. Data for days 18 to 30 are used only for qualitative comparison and discussion.

The extent of marks recorded per tile usually increased from one record to the next, but sometimes decreased depending on weather conditions. Dry weather reduced the amount of water the urine retained thus reducing the visibility of the marks, some of which became undetectable. In extreme humidity, however, water in the air impregnated the salts and other chemical components of the dry urine and made the marks highly conspicuous.

Comparisons of the extent of the tile surface urine marked were conducted using non-parametric matched-pairs tests (Meddis, 1984). Data from different pens were not related, but counts within an enclosure depended on the same pair of rats and reflected the cumulative urine marking since the establishment of the group. Thus data were ranked within each enclosure for every record taken (day of record as dependent variable and pen as independent variable). The mean rank was computed for each tile over the 18 day period of study. The mean rank for each tile was, in turn, used to compute the mean rank of the different tiles at each area tested (boundary versus central area), to take into consideration the fact that both areas had different sizes. These values were then ranked 1 or 2 within enclosure to conduct the test. Ranks were used instead of the data on the extent of surface marked because the mean rank depends only on the mean order of its values, whereas the mean marked surface is likely to be affected by single extreme values.

Another advantage to using non-parametric Meddis ANOVAs is that specific hypotheses, such as those in the following analysis, can be tested.

The pilot test suggested that the density of urine marks may be higher along the boundaries of an enclosure. Hence, the data were analysed using a specific test for the following questions:

-Do rats deposit more urine marks around the boundaries of an enclosure than in the central area?

The coefficients for the ANOVA were thus:

	Boundary	Central area
λ	2	1

4.2.3.3. Results.

As expected rats deposited a greater coverage of urine marks at the boundaries of the enclosure ($Z=2.65$, $P<0.01$, see 3.1) than elsewhere (figs. 4.1, 4.2, 4.3 and 4.4).

Three additional enclosures studied the following year showed similar patterns in urine deposits. Differences in the density of urine marks between boundaries, nest and centre of the enclosure were very apparent, but no records were taken.

The density of marks in colonies 6 and 7 was far greater than that in colonies 4 and 5 (mean percentage of the area marked in the last count after 18 days, colony 4=16.59 ± 1.56 %, n=36; colony 5=0.73 ± 0.38 %, n=36, colony 6=45.30 ± 3.31 %, n=36; colony 7=31.79 ± 3,72 %, n=36). However, no statistical analysis was attempted. In colony 5, where the female had died, the extent of marks was close to 0 for most of the period of study. The level of urine marking in colonies 4 and 5 increased steadily and reached rates of urine marking in the additional period of study (day 18th to

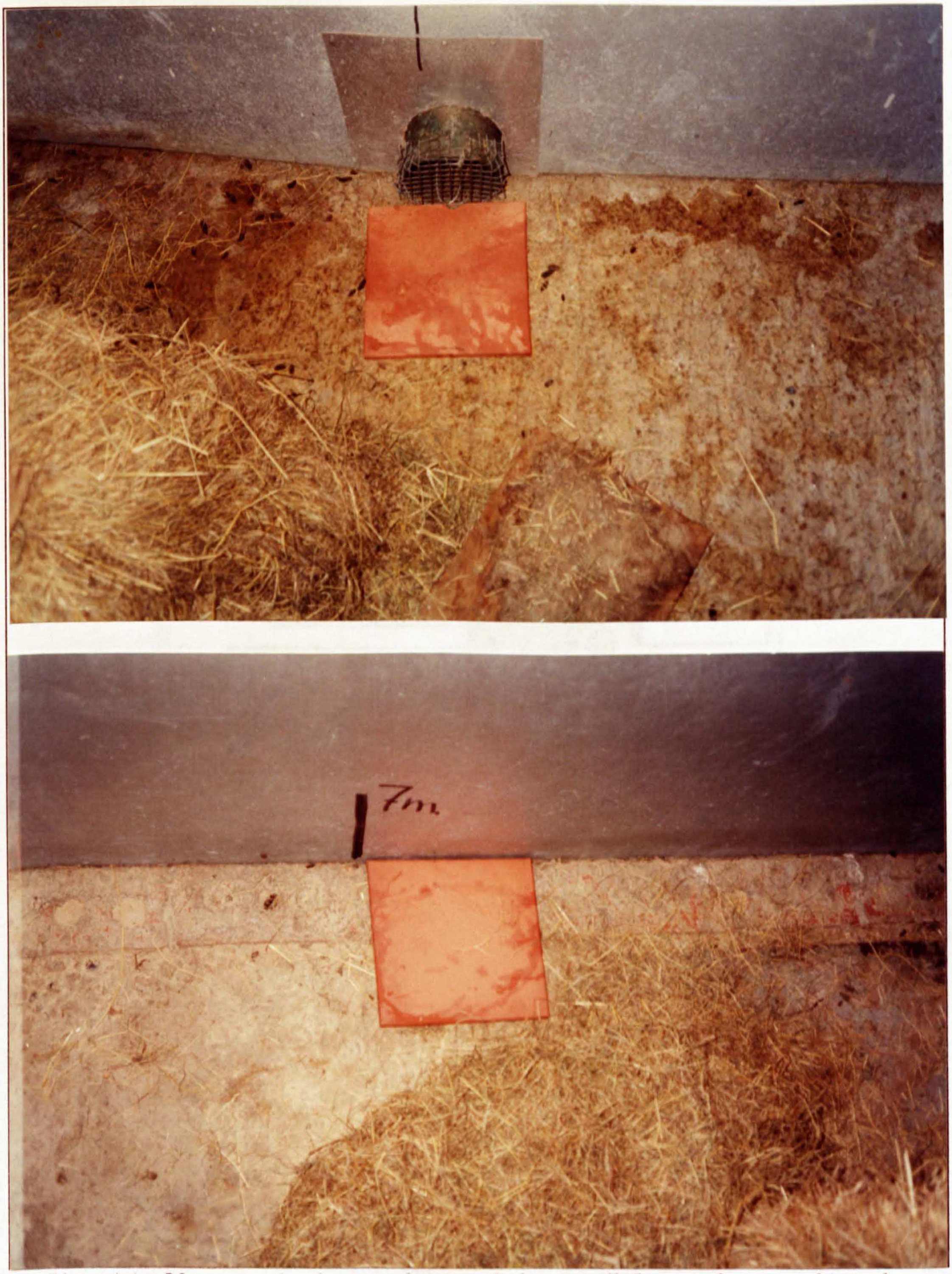


Plate 4.1. Urine marks deposited on a tile by a wall (lower photograph) and a tile at one end of a pipe communicating both enclosures (upper photograph). Rats urine marked overnight the tile at the pipe almost entirely, while only a fraction of the wall tile surface was covered by urine (20% mean percentage on tiles by the wall in the colony marking most, see text).

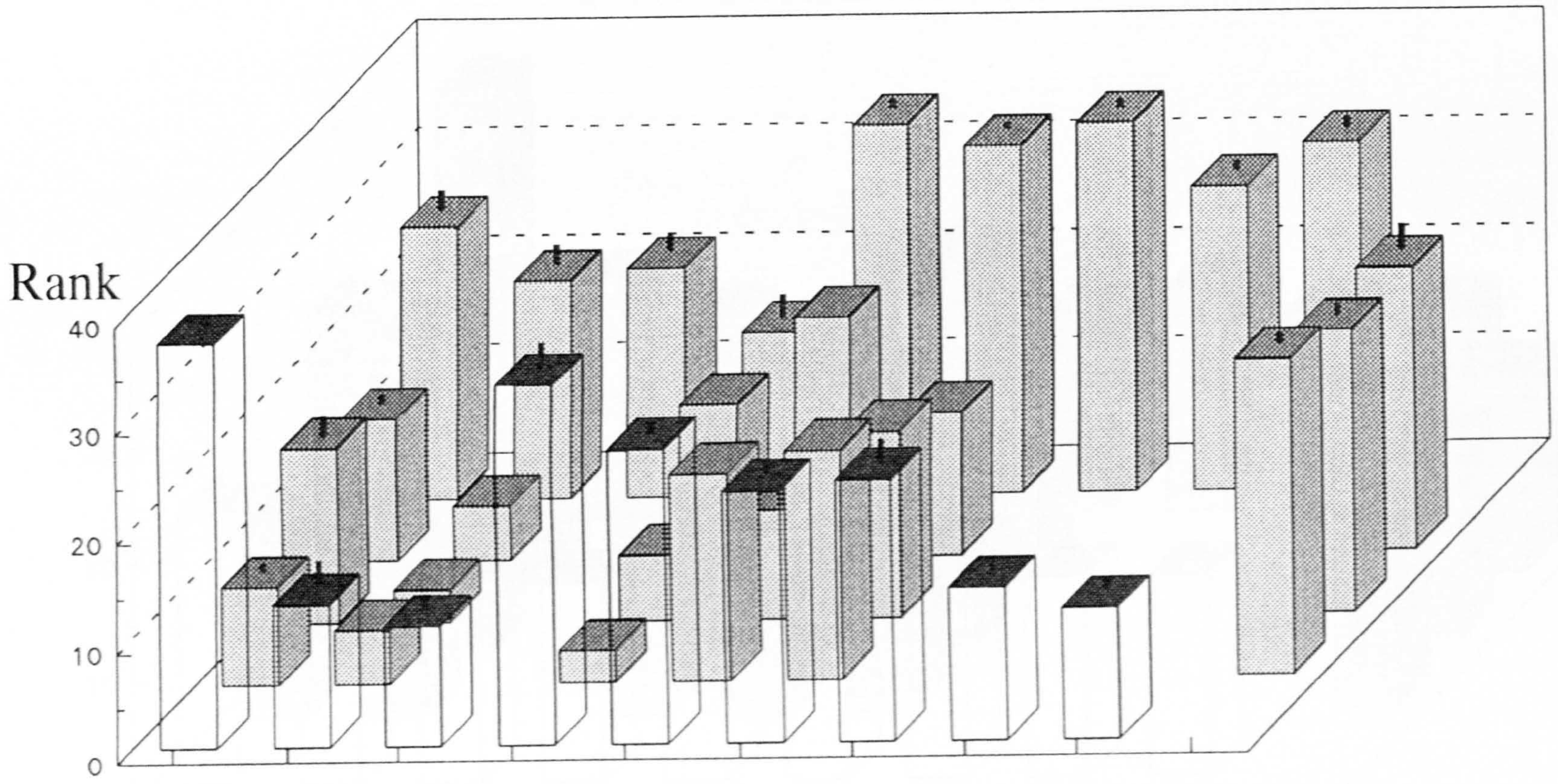


Fig. 4.1. Mean ± SE rank of urine distribution in colony 4. The empty area corresponds to shelter.

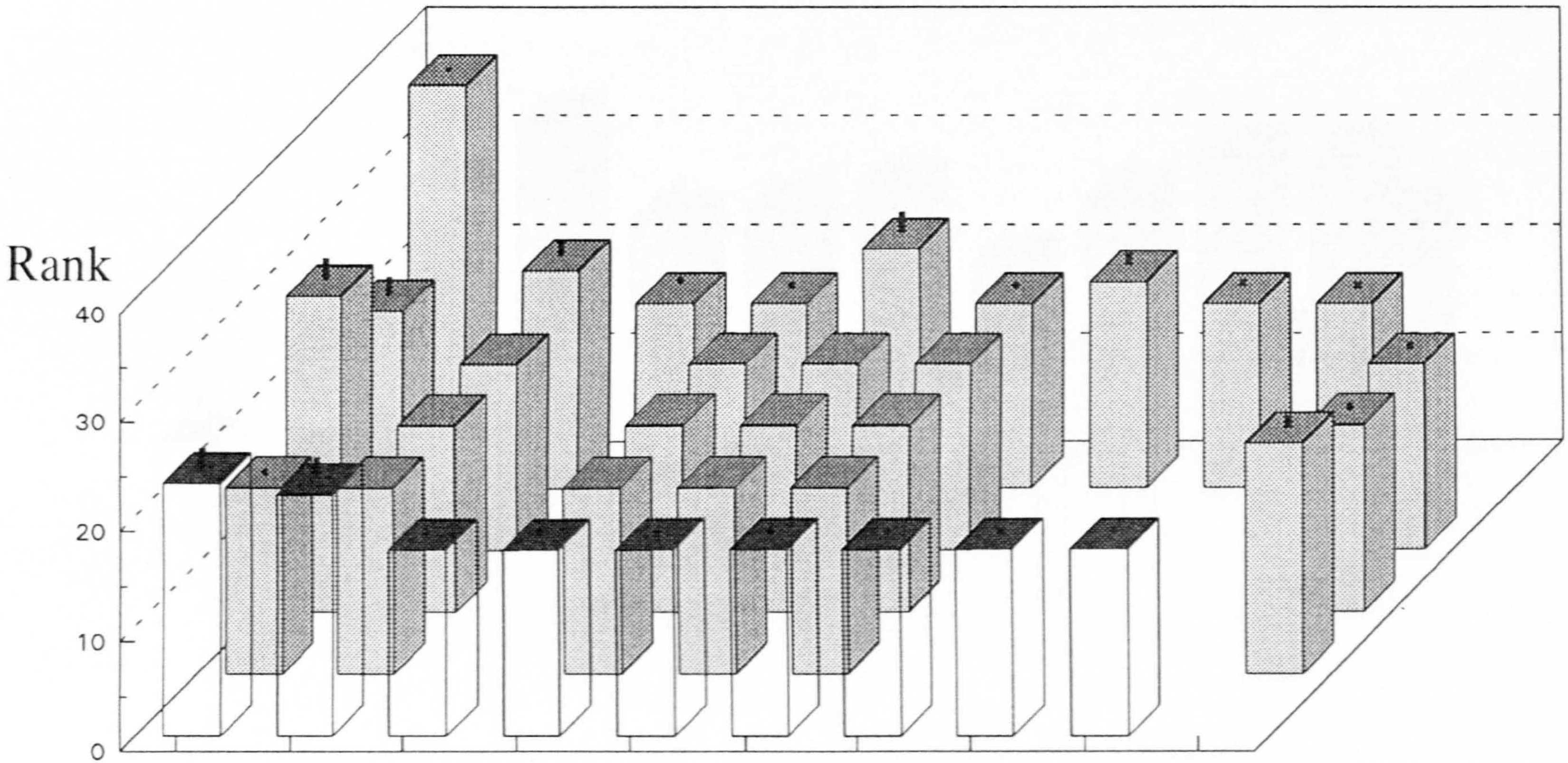


Fig. 4.2. Mean ± SE rank of urine distribution in colony 5. The empty area corresponds to shelter.

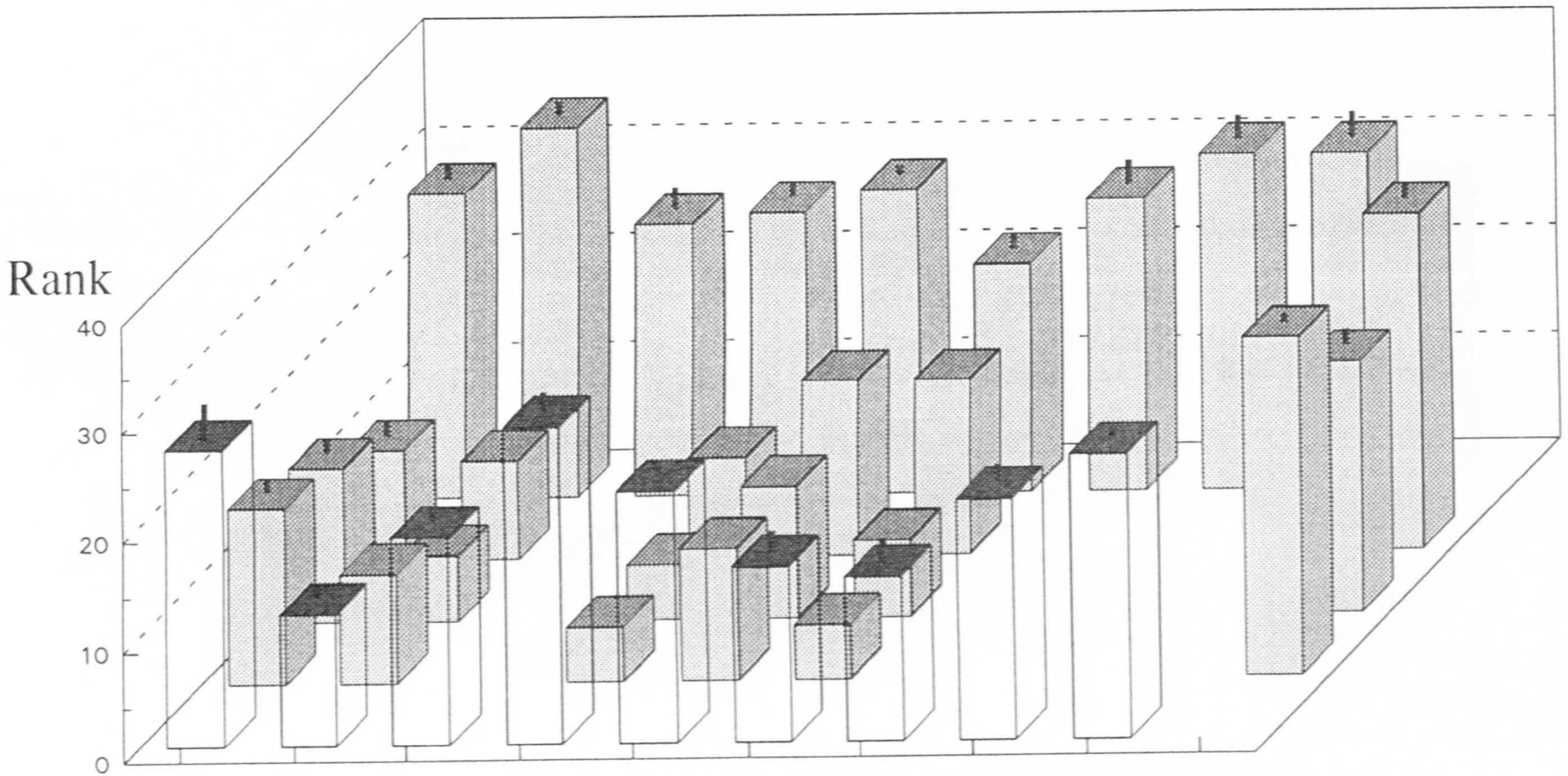


Fig. 4.3. Mean ± SE rank of urine distribution in colony 6. The empty area corresponds to shelter.

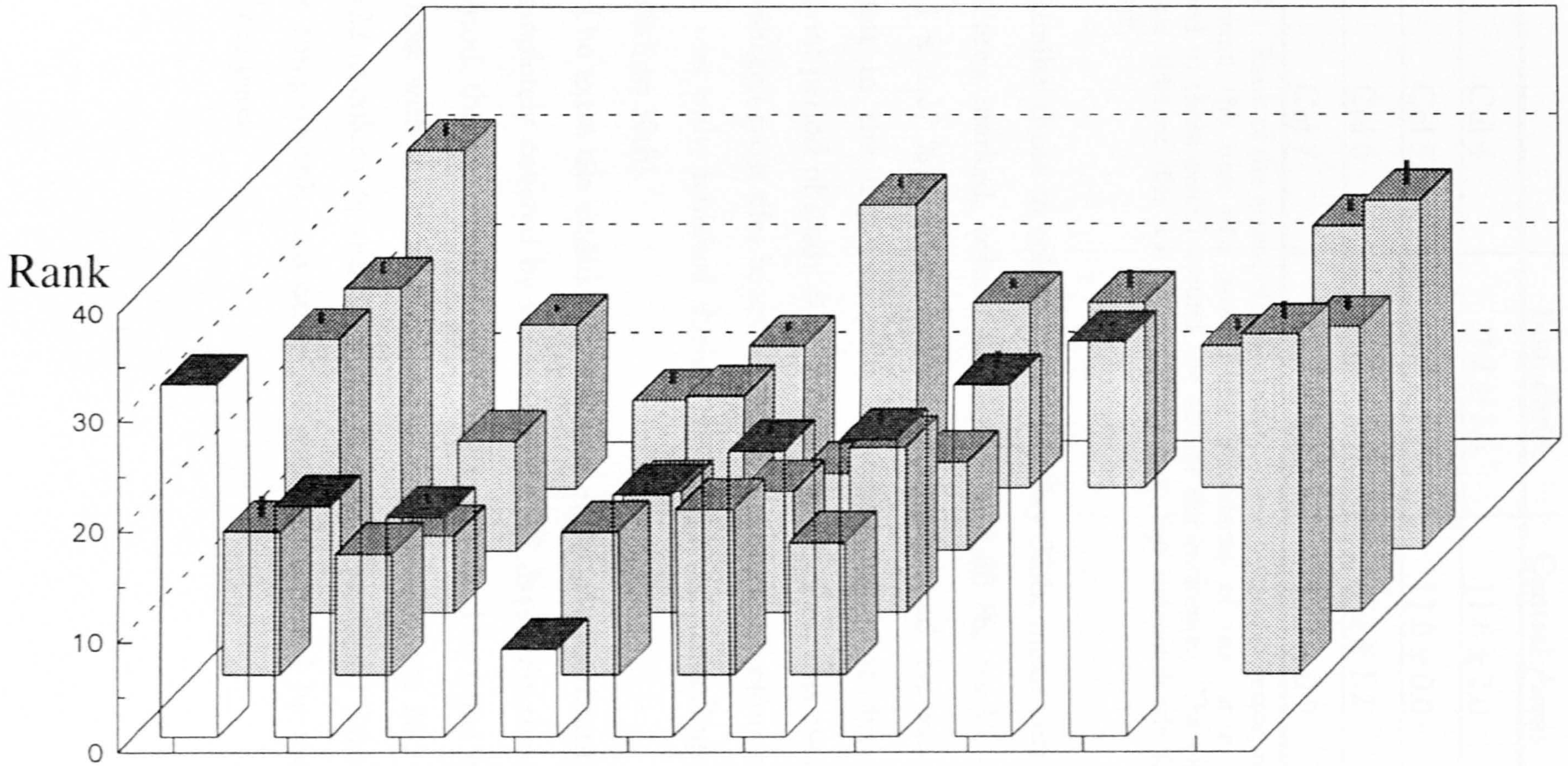


Fig. 4.4. Mean ± SE rank of urine distribution in colony 7. The empty area corresponds to shelter.

	Boundaries	Central Areas
Col 4	23.2 ± 1.6	11.6 ± 2.0
Col 5	20.7 ± 1.2	17.0 ± 0.0
Col 6	24.2 ± 1.4	9.2 ± 1.2
Col 7	23.4 ± 1.7	11.1 ± 0.9

Table 4.1 Rank of the extent of urine marks (mean ± standard error, n=9) on tiles around the nest and around the boundaries of the rat enclosures compared to those placed around the rest of the enclosure. The extent of marks on different tiles was ranked every two days independently for each enclosure.

30th) similar those in colonies 6 and 7 (day 30th, mean percentage of the area marked, colony 4=16.66 ± 1.80 %, n=36; colony 5=7.43 ± 1.27 %, n=36). Higher marking around the boundaries and nest in the first two pens was more apparent during the additional period of study than before. In all cases, rats eventually urine marked most tiles to some extent, although in colonies 4 and 5, this was only achieved during the additional recording period (days 18th to 30th).

The extra tile added to either end of the communicating pipe was completely covered by urine after only 8 days. At the end of this period, the mean percentage of surface covered by urine on wall tiles (those with the highest density of urine marks) was 20.5 ± 2.5 %, n=22 in colony 6, and 8.8 ± 2.0 %, n=22, in colony 7 (see plate 4.1 for comparison). No other tile was fully covered by marks in any of the pens.

4.2.3.4. Discussion.

As discussed in section 2.1, tiles were introduced as a method of sampling the urine marking on the floor, and to allow the exchange of odours between enclosures. It could be argued whether or not the density of marks on them was representative of urine mark density on the floor. The tiles protruded about 1 cm above the floor. As a consequence, they may have attracted extra marking. However, this does not necessarily mean that tiles were not representative of floor marking. Fresh urine marking was also detected on the bare concrete floor. In addition, raised edges are a normal feature of most environments. Furthermore, the distribution pattern of urine observed in undisturbed colonies of rats where there were no tiles present was similar to that in the pens where tiles had been introduced (section 4.2.1 and 4.2.2). In addition, rats did not seem to change their routes in order to mark the tiles. Thus, it appears unlikely that the observed pattern of urine mark deposition was altered by attraction to mark the tiles in preference to the surrounding floor. There were logistic difficulties in using alternative settings. Perhaps the ideal set up would have been either to cover the floor entirely with tiles or to insert them into the floor surface so that they did not protrude, but these alternatives were impossible to carry out. The former was prevented by cost-benefit considerations and the latter by the impracticability of altering the enclosures in such a way, and because the tile insertion points probably would have accumulated urine.

Rats eventually urine marked all tiles in the enclosure, though rats in pens 4 and 5 took longer to mark them than those in pens 6 and 7. There does not appear to be an obvious explanation for these differences. Tiles were thoroughly washed, rinsed and dried

before the establishment of the second batch of colonies. The tiles, nevertheless, seemed to absorb urine and thus, rats in the second batch may have been responding to scent cues left on the tiles. Laboratory rats investigate and mark conspecific odours more than clean stimuli (Brown, 1975, 1977, 1985c, 1991, 1992; Hopp and Timberlake, 1983; Birke and Sadler, 1984). An alternative explanation could be that urine marking odours released in the air by one colony marking strongly could stimulate similar levels of marking in the neighbouring colony. Hence, one pair of rats in the second batch might have been prolific markers by chance and have induced a similar level of marking in the other pair. Regardless of the actual level of urine deposition, the most important fact was that the distribution of urine marks was basically similar in all colonies.

The density of urine marks maintained on tiles located around the boundaries was much greater than those sited in central areas. This finding is consistent with other studies where laboratory rats kept in small cages were used (Richards and Stevens, 1974; Hopp and Timberlake, 1983; Peden and Timberlake, 1990). Rats seemed to be more active around these highly marked areas, which suggests that they might be the urine trails reported in the literature (Telle, 1966; Boice, 1977), but further tests are needed to prove it. It is particularly suggestive that the tiles at either end of the pipe allowing exchange of odours were completely covered by urine when none of the rest were. Indeed the mean percentage of tile surface covered by urine on other tiles by the walls was below 25%. It seems likely that the tile by the pipe was heavily marked in response to the odours coming from the neighbouring pen.

Although suggestive, these results do not demonstrate that urine marks play a role in orientation or social communication.

This uneven distribution of marks might be due purely to incidental deposition, corresponding directly to their frequency of visits. Conversely, rats may have been actively marking areas that they visited less frequently than the density of marks suggested (as happened with the latrines discussed in chapter 3). Even if the pattern of urine deposition is just a by-product of the daily activity of the rats, this would not indicate whether the urine marks play a role in orientation or communication.

It is suggestive that rats deposit more urine in areas or pathways that they frequently use. Urine marks might signal the degree of risk in a site because areas containing a lower density of urine marks were generally open areas which were usually riskier areas to move around, whereas those containing a higher density of urine marks were either protected by a wall or they were close to the nest. Alternatively, these urine trails may carry information regarding food, like the scent trails found by Galef and Buckley (1996).

4.3. Response to clean tiles.

4.3.1. Aims.

If urine marking plays a role in orientation and/or social communication, rats should detect and respond to manipulations of the urine background. An experiment was conducted to assess whether rats detect such manipulations by presenting rats with a noticeable manipulation of the odour background, i.e. the introduction of a clean tile which constituted a 'gap' in the odour profile. The test had two goals:

-To assess whether rats detect "gaps" in the background of urine marks (areas of experimentally-reduced urine density).

-To determine whether rats maintain the observed distribution pattern of urine marks (see previous section).

If rats detect and 'fill in' gaps in the pattern of urine marks it may suggest that the pattern of urine marks encodes information useful to the rats. A mistake during a pilot experiment (section 4.6) suggested that rats might tend to fill the gap in the background of urine marks if the trial lasted long enough (e.g. a whole night, although they failed to mark over the first 1 hour period).

4.3.2. Methods.

Rats were presented with a choice test between a clean tile and a familiar marked tile placed (as a control) in their already urine marked home pen. The experiment was conducted using colonies 4, 6 and 7. In colony 5, where the female had died, the male scarcely marked the tiles, except at the end of the experimental period. Thus, there was no background of urine marks during the period when the test was being carried out in the other colonies so the experiment was not carried out in this colony.

The experiment was carried out in two areas of the enclosure, i.e. it had two treatments:

-Treatment one. Test tiles were placed by a wall, where the density of urine marks was higher.

-Treatment two. Test tiles were placed in the centre of the enclosure, where the density of urine marks was lower.

Nine replicates were carried out per enclosure and treatment. Urine marks were measured using a 0.75 cm^2 grid (section 2.6). The location of the clean and control tiles was chosen at random. Both experimental and control tiles were placed by the same wall and occupied consecutive sites (i.e. they were 1 m apart). In colony 4,

native tiles by the wall near the drainage channel were not marked. As there was no background against which clean tiles could be detected, treatment 1 was conducted only by the remaining three walls. To standardise the procedure, the wall by the channel was excluded from treatment 1 in other pens even though these had a background of urine marking by this wall.

The tiles used were the quarry tiles described in chapter 2 (section 2.6). Each trial was set up in the evening (about 2100 h) and left overnight for the rats to respond. A trial lasted a variable number of hours (12-14 h), although this variability is unlikely to have affected the experiment because rats were scarcely active in the last 3-5 hours of the trial (after 0600). Native tiles replaced by a clean tile were removed and left on an inclined sheet of metal on top of the enclosure wall (fig. 2.2). The familiar marked tile was lifted as a control manipulation and returned to its original position. After each trial the clean tile was removed and the native tile replaced back in its original position. The extent of the surface marked with urine was recorded on both clean and control tiles, prior to and after the experiment. The previously clean tile was washed after the test, scrubbed with liquid detergent and water, and then left to dry for at least one day before reuse. New tiles were used as clean tiles as often as possible, although new and re-used tiles were washed using the same procedure to standardise any possible odour cue.

The results were analysed using two-way non-parametric ANOVAs (Meddis, 1984) to assess the effects of position and colony.

Firstly the density of urine marks on the familiar control tiles was assessed to corroborate that urine marking at the boundaries, as found in section 4.2, was higher than marking in the centre of the

enclosure. Urine marking on tiles used as controls both by the wall and in the central areas was measured prior to experimental manipulation. Because the test by the wall started a few days before that in the centre, results were ranked within each pen instead of matching controls by the wall with those in the centre. In addition ranking within each pen was necessary because the test consisted of repeated measures. A two way non-parametric ANOVA tested the specific prediction that urine marking on familiar tiles by the wall was greater than that on tiles in the centre. The coefficients were:

	colony 4	colony 6	colony 7
tiles by the wall	+1	+1	+1
tiles in the centre	-1	-1	-1

The urine marking response to clean versus familiar marked tiles was also analysed using a two way non-parametric ANOVA. Results were ranked 1 or 2 within each day and enclosure. A specific test was conducted to test whether the rate of urine marking in response to clean tiles (the 'gap' in the urine background) was greater than that in response to familiar (marked) control tiles. This response was expected in both treatments. Thence, the coefficients for the test were:

	colony 4	colony 6	colony 7
experimental	+1	+1	+1
control	-1	-1	-1

This test, however, presents a problem: rats could either be marking the clean tile at a higher rate than the control, or they could be marking both at the same rate, but the new marks on the familiar marked tile were obscured by old marks. Both possibilities would yield the same result of apparently higher marking rate on the clean than on the familiar tile.

To discriminate between these possibilities, the real rate of urine marking had to be estimated and then compared with that on clean tiles. The '*real rate of urine marking*' comprises all the marks deposited, whether on top of previous marks or on unmarked parts of a tile. The urine marking rate was computed as the extent of urine marks after each test minus the extent of urine marks before the test $(m_a - m_b)$. This crude estimate of the new marks, however, does not take into account that some of the marks newly deposited will be deposited on top of old marks. The real rate of urine marking was estimated as the proportion of the clean surface marked during each test (i.e., the real rate of marking was $[m_a - m_b] / [T_s - m_b]$, where m_a is the surface marked after the trial, m_b the surface marked before the trial, and T_s the total tile surface). Thus if, for example, 20% of the clean surface was marked during a particular trial, we assume that another 20% was deposited on top of old urine marks (which was unrecorded) and hence, 20% of the total tile surface had been covered with new marks during that trial. It is important to notice that this estimate is based on the assumption that new marks are deposited *at random*. This assumption is discussed in section 4.3.4. As the proportion of marks on familiar marked tiles was never larger than 50% no trial had to be discarded for having a clean surface too small to detect new marks.

Results are given as means per colony ± SE.

4.3.3. Results.

As expected, the density of urine marks on familiar tiles used as controls by the wall was higher than on equivalent tiles in the centre of the pen (percentage of surface covered: $Z=4.56$, $P<0.001$; controls by the wall 17.83 ± 4.35 %, n=3; controls in the centre 4.67 ± 2.02 %, n=3).

Also as expected, the percentage of surface covered by marks on clean tiles was greater than the overnight increase in surface marked (m_a - m_b) on familiar marked controls in both treatments (percentage of marks on clean-surface-marked-overnight/total-tile-surface: tiles by the wall; $Z=5.66$, $P<0.001$; clean tile 15.53 ± 6.46 %, n=3; familiar marked control 3.95 ± 0.73 %, n=3; tiles in the centre of the pen: $Z=3.72$, $P<0.001$; clean tile 7.16 ± 3.21 %, n=3; familiar marked control 2.03 ± 0.88 %, n=3).

The rate of marking on the clean tile was still higher than the real rate of marking on the familiar marked control tile both for tiles by a wall ($Z=5.12$, $p<0.001$; clean tiles, 15.53 ± 6.46 %, n=3; familiar marked control tiles=4.94 ± 1.09 %, n=3) and tiles by the centre ($Z=3.25$, $p<0.001$; mean percentage ± SE, clean tiles, 7.16 ± 3.21 %, n=3; familiar tiles=2.16 ± 0.92 %, n=3; see fig. 4.5).

For tiles by the wall, nevertheless, the familiar marked control tiles showed a larger extent of surfaced covered by marks (old and new) after the test than clean tiles (effect of tile type, $H_{1df}=16.36$, $P<0.001$; mean ± SE, clean tiles=15.53 ± 6.46 %, n=3; familiar tiles=21.77 ± 4.79 %, n=3) but it is important to note that this was only found to be the case in two colonies (interaction between tile type and colony $H_{2df}=6.98$, $P<0.05$, see fig. 4.6). The other colony marked the familiar control tile slightly more than the clean tile, but

this difference failed to achieve statistical significance as a result of high variability in the response.

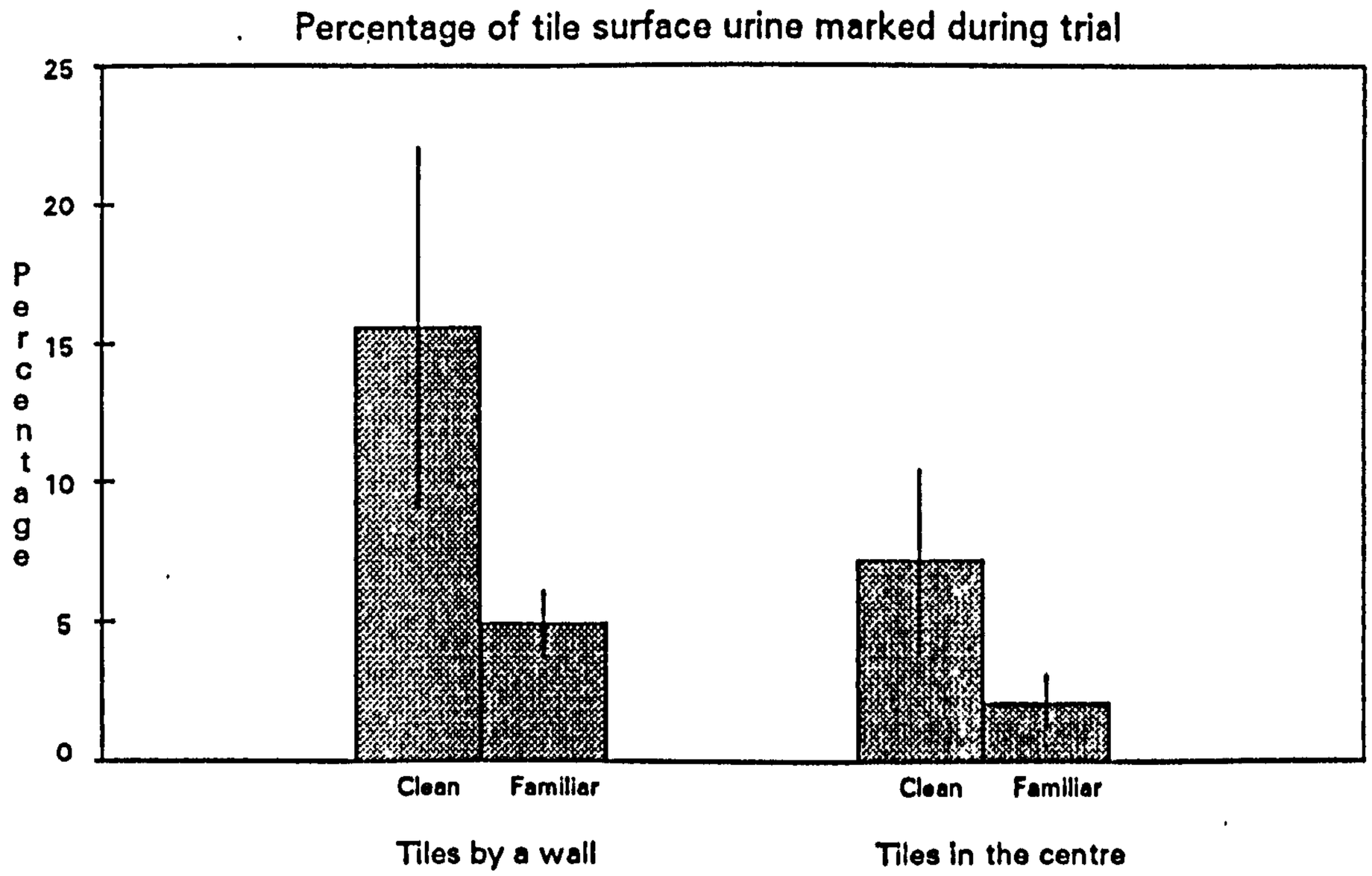


Fig. 4.5. Comparison between the extent of urine marks deposited overnight on a clean surface and the estimated extent on a familiar marked tile (mean ± standard error of the percentage) in two sites: tiles by a wall and tiles in the centre of the enclosure. The extent of overnight marking on the familiar tile was estimated as the proportion of clean surface marked in that night. Rats marked the clean tile more than the familiar marked control (but see discussion).

In the centre of the pen, clean tiles achieved a percentage of marked surface similar to that on familiar marked controls (effect of tile type, $H_{1df}=2.05$, ns; clean tiles 7.16 ± 3.21 %, n=3; familiar tiles 6.69 ± 2.56 %, n=3). Not all pens showed the same trend: the proportion of marks on the clean tile in colony 7 was greater than that on the familiar tile, whereas the trend was reversed in colonies 4 and 6 (interaction between tile type and colony, $H_{2df}=2.05$, ns; see fig. 4.7).

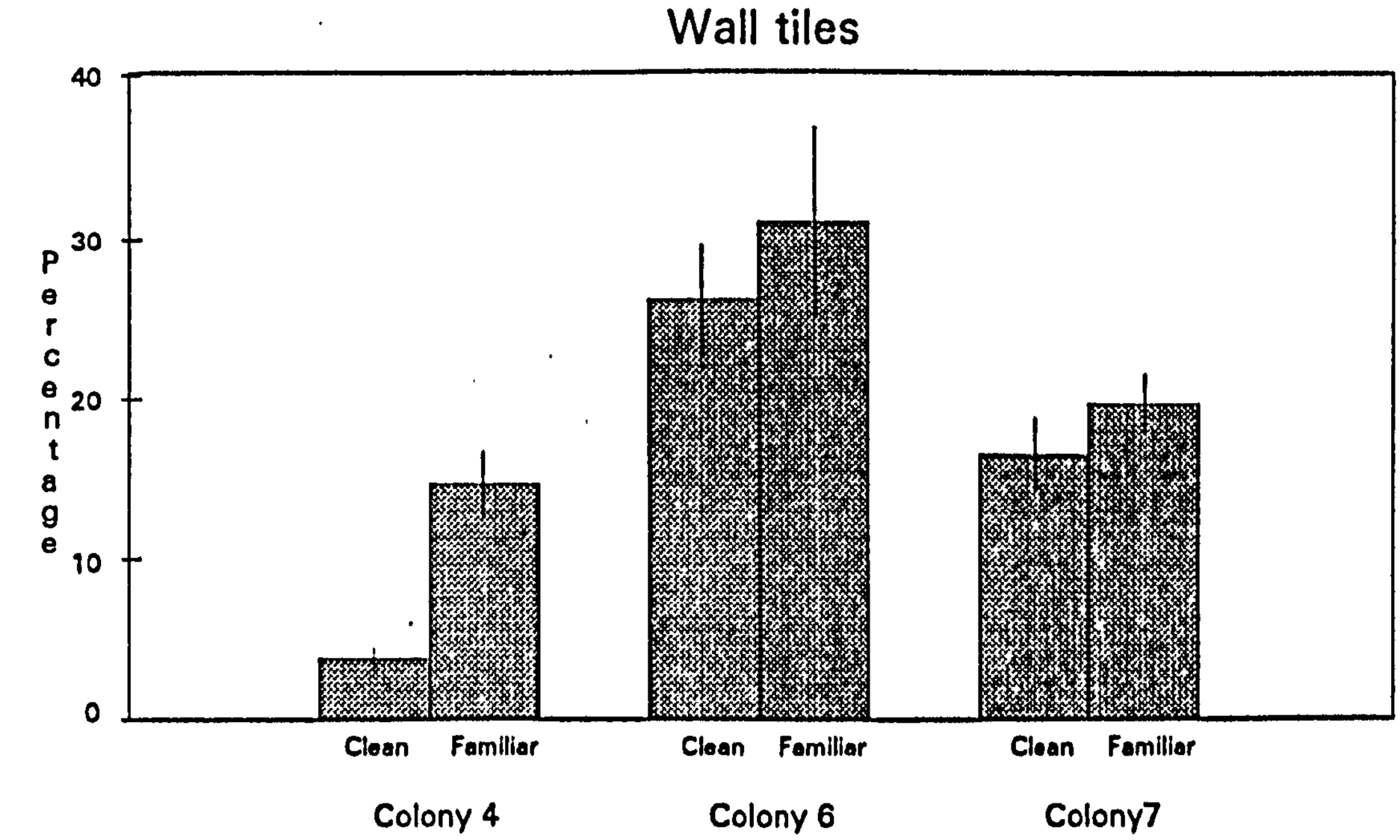


Fig. 4.6. Final extent of urine marks (mean ± standard error of the percentage) in a previously clean tile (first row of each group) versus a familiar tile (second row) over all trials conducted by a wall. Colony 6 did not show a significant difference between the greater extent of marks on the familiar tile compared to the previously clean tile.

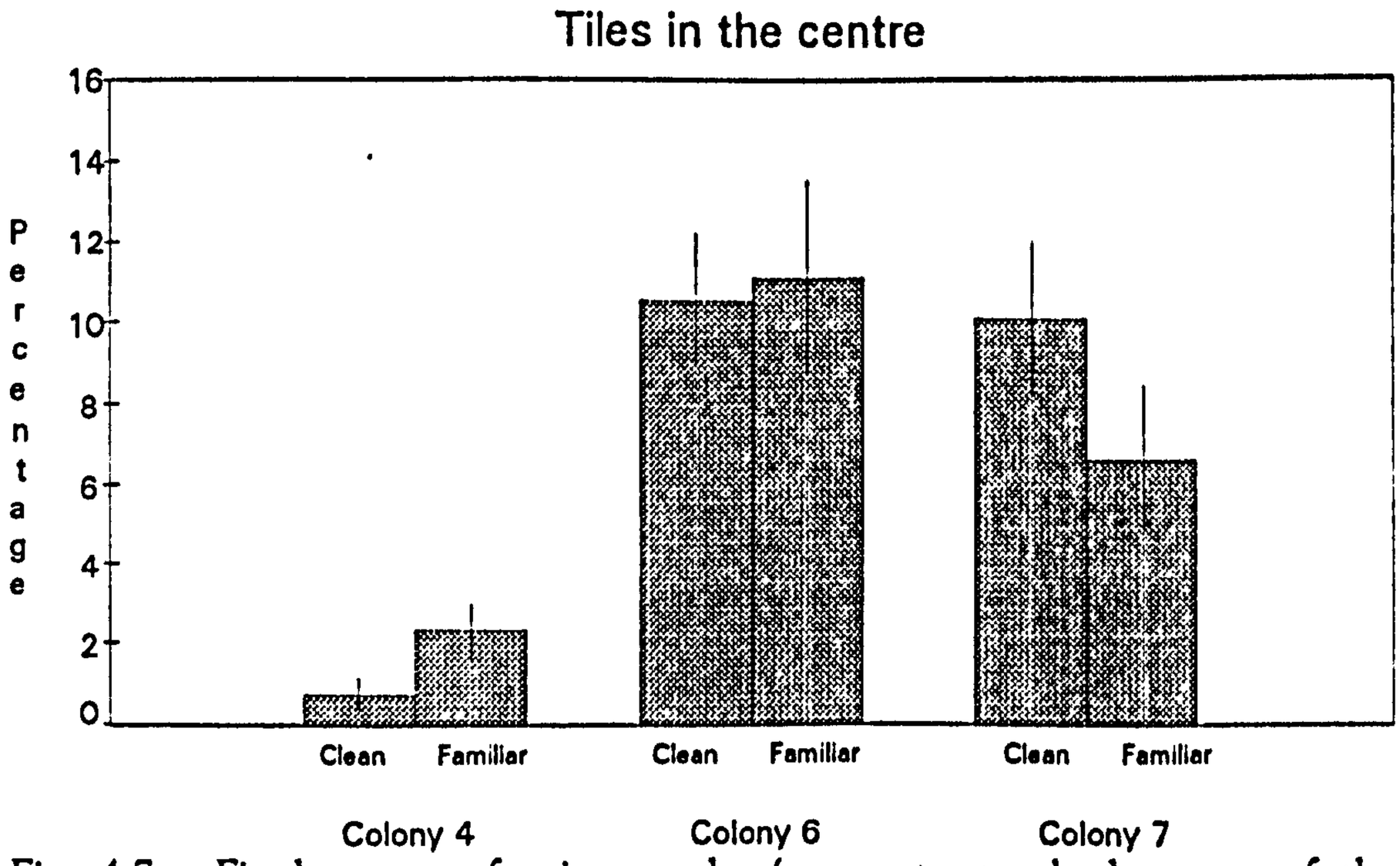


Fig. 4.7. Final extent of urine marks (mean ± standard error of the percentage) in a previously clean tile (first row of each group) versus a familiar tile (second row) over all trials conducted in the centre of the pen. The final extent of the clean tile in colony 7 was higher than the familiar, whereas colonies 4 and 6 showed an opposite difference between both types of tile.

4.3.4. Discussion.

The comparison between familiar marked controls at the centre of the pen and those by the wall agreed with the distribution pattern of urine marks found in section 4.2, with a greater density of urine marks by walls than in central areas.

The rate of marking was far greater on clean tiles than on the familiar and already marked tiles, even when correcting for over-marking. This means that rats responded to the gap in the established odour profile by increased marking, as if they were trying to reinstate the pattern in these sites.

It is important, though, to discuss some limitations of the estimate of the real marking rate. The method used assumes that urine marks are deposited at random. Therefore the same proportion of new marks are placed on the clean portion of the tile (which can be measured) and on the marked portion (which cannot). There are, at least, two reasons why this assumption may not hold:

-Rats may prefer to mark certain parts of the tile (e.g., the edges).

-Urine marks may be more likely to be deposited over old marks (counter-marking).

In both cases most of the new marks would be deposited over old marks and therefore, even the estimate of the real marking rate would be smaller than its real value. Thus, the difference between the marking rate on the clean, and on the familiar tiles would appear to be larger than it is.

Urine marks on the tiles were not mapped and their distribution was not recorded in any form. The surface of the tile

covered with urine marks on each tile was recorded as a single figure. However, the distribution of the marks can be described qualitatively and this may help in discussing both objections:

-The tiles placed by the wall were sited with one of their edges touching the wall. Among these tiles, those sited near the nesting area showed a random distribution of urine marks with no apparent pattern. In contrast, the tiles by a wall away from nesting areas showed streaks of urine usually crossing the tile along the side by the wall. Rats seemed to mark when they crossed the tile by the edge next to the wall. There was also a higher density of urine marks around the edge on the half of the tile facing the centre of the enclosure.

-On tiles near to the hay stack but away from the walls, the distribution of urine marks appeared to be random with no apparent pattern.

-The tiles in the centre of the enclosure showed a concentration of marks around their edges, though there were also urine marks on the centre of these tiles.

It seems to follow that marks tended to be deposited on certain parts of the tiles, and thus, that the estimated rate of urine marking on controls might be smaller than the real marking rate. However, several facts suggest that the marking rate on clean tiles was, nonetheless far greater than that on control tiles:

i) The rate of urine marking on clean tiles was three times greater on average than that on familiar marked controls. Rats would have to mark with a 66% preference over old marks for both rates to be equal (that is, 2/3 of the real marking rate should be hidden by old marks). A bias of this extent would have been obvious to the observer. Whenever marks could still be detected as

freshly deposited, they did not appear to be especially sited over old marks. Although new marks on top of old marks were not recorded, they could frequently be distinguished as moist, bright patches over dried marks. The impression of the general pattern of overnight marking was that urine marks were more common on the clean than on the familiar marked tile.

ii) The total extent of the surface marked on clean tiles after the test was sometimes larger than that on familiar marked tiles. It occurred in 6 out of 27 (22%) tiles by the wall and in 9 out of 27 (33%) tiles sited in the centre of the pen. The differences were not small: 26% marking on the clean tile versus 10% on the control in one case, 10% versus 1% in another, 25% versus 10% in another and so on. This corroborates the strong impression than urine marking on the clean tiles was clearly greater than that on familiar tiles.

Urine marking may also have been triggered by manipulation of the tiles. Thus the rate of marking on the familiar tile may have been higher than on other familiar tiles nearby which were not manipulated. Perhaps in a more controlled situation where no artificial odour from gloves could be transferred to tiles, the differences between clean tiles and familiar controls would be even larger.

Wild rats, thus, detected gaps in the background of urine marks. They responded by greatly increasing the rate of urine marking in that site. This result contrasts with some reports in the literature, where clean stimuli attracted less marking than the rat's own urine marks (Brown, 1975, 1977, 1985c, 1991, 1992; Hopp and Timberlake, 1983; Birke and Sadler, 1984). However, the cited tests were carried out in clean unfamiliar arenas, where there was no

background of urine against which a clean stimulus would stand out. In contrast, Hurst (1987), in a study conducted on free-ranging populations of wild mice, found that clean stimuli triggered more investigation and urine marking compared to the response towards unfamiliar urine marks. Mice seem to create a urine marked background against which any change can be detected (Hurst, 1989, 1990a, 1990b, 1990c).

The extent of surfaced marked overnight on clean tiles was similar to that of surrounding tiles. Although the statistical analysis failed to reach a significant level of similarity for tiles by a wall, figures 4.6 and 4.7 show a similar percentage of marked surface after the test on clean and control tiles sited either by a wall or in the centre of the enclosure.

In summary, this experiment indicated that urine marking of the home range was not just purely incidental. Rats detected the presence of clean tiles against a familiar marked background and responded by increasing their rate of urine marking, covering the clean tiles to a similar extent as urine marks already present on surrounding tiles. Urine thus must convey some information for the rats, either in itself or in its distribution pattern.

4.4. Sex bias in urine marking.

An indication suggesting that scents are implicated in communication is that males and females mark at different rates (Ralls, 1971; Thiessen and Rice, 1976). Comparison of urine marking between the sexes might suggest whether urine marking is used in social communication. If urine marking serves as a status badge, top ranking individuals (usually the largest males) should deposit more urine marks than other colony members. Physiological

differences in the production of urine might be needed to sustain such a rate of urine marking. If urine is used by females as a sexual attractant, they should mark at a higher rate when receptive than when in anoestrus or dioestrus. However, a sex bias in urine marking, although suggestive, would not be conclusive, as the difference in the rate of urine production might be due both to differences in size between males and females and also due to differences in their metabolic rate.

It is important to note that orientation and social communication roles are not mutually exclusive. A dominant individual may mark its home range thoroughly as a status badge, but the pattern of deposition may also serve for orientation, indicating to the resident when he is leaving his home range.

The literature on rats appears to be contradictory about sexual differences in marking rates. To unravel the role that urine marking seems to play in a competitive situation, individuals need to be tested in their familiar enclosure, where they are likely to defend their territory. Most studies, however, have been performed on laboratory rats in clean unfamiliar arenas (for example: Carr, Loeb and Dissinger, 1965; Carr, Krames and Costanzo, 1970; Carr, Wylie and Loeb, 1970; Carr et al., 1976; Brown, 1975, 1977, 1985c, 1986c, 1991, 1992; Birke and Sadler, 1983, 1984; Birke, 1984; Brown, Singh and Roser, 1987, to mention but a few). In these, the lack of a familiar olfactory background prevents scent matching, precluding the association between animal and territory, and impeding the expression of territoriality. Among all the reports, only Price (1977) tested individuals in their home cages.

Differences between studies of marking by rats may also arise from differences in methodology. Both Price (1977) and Brown

(1991) recorded urine marking as the surface covered by marks. They found that males marked a larger extent of a stimulus object than females. Other researchers (Birke and Sadler, 1984; Lee, Mitchell and Adams, 1984; Peden and Timberlake, 1990) recorded marking as *the act* of urine marking, i.e., the frequency of marking. Lee, Mitchell and Adams (1984) and Peden and Timberlake (1990, for urine marking at the burrow entrance) indicated that 'females mark more than males', apparently in contrast with Price (1977) and Brown (1991), while others found no such sex bias (Birke and Sadler, 1984; urine marking of objects: Peden and Timberlake, 1990). However, because these latter reports refer only to a greater frequency of urine marking by females, the lack of agreement is only apparent, because females may mark more often than males but deposit smaller amounts of urine, resulting in the larger extent of male marks reported by Price (1977) and Brown (1991).

The lack of agreement between authors that reported marking as the frequency of this act is not surprising because all of them recorded the event of marking as perineal dragging or crossing over the stimulus object. It is very difficult to discern, even from a short distance, if the individual crossing is dragging the perineum, or, in that case, if the rat actually urine marked. By measuring urine marking as the extent of marks deposited per visit and trial and not as the act of dragging the perineum, my study was intended to provide clearer results than those reporting frequency of marking.

Most hypotheses predicting a bias in urine marking would expect males to mark more than females. Apart from physiological differences derived from a greater male body size, a greater marking rate by males might be explained if urine plays a role in social

communication. This could be either in sexual communication or competitive advertisement.

Males may be expected to mark more than females if urine serves to attract mates because females are receptive for a short period of time, whereas males can mate at any time. As a result, females should advertise intensely (marking perhaps more than males) while they are receptive. Because they are either pregnant or lactating most of the time, their rate of marking to attract mates should be small for most of the time. In contrast, as males are predisposed to mate at any time, they should usually mark more than females. However, males do not always treat females as possible mates. On the contrary, sometimes females are considered as competitors and thus males sometimes attack intruder females (section 1.4.5.1).

More urine marking by males is also predicted by a competitive advertisement hypothesis (e.g. if urine signals who is the dominant individual, warns intruders that the area is occupied, etc.). In both cases, only the individual most competent at fighting should mark. Both sexes might mark to compete with individuals of their same sex (section 1.4.7.2.5). However, if intersexual competitive advertisement occurs in rats, the female is unlikely to advertise more strongly than males because: i) male rats are usually larger than them; ii) females rats are reluctant to attack males larger than themselves (Flannelly and Flannelly, 1985); iii) intruder males retaliate if attacked by a female, but never if attacked by a male (Flannelly, Flannelly and Lore, 1986); iv) the top ranking individual in captive or free-ranging wild rats is always reported to be a male (Barnett, 1958; Robitaille and Bovet, 1976; Berdoy, Smith and Macdonald, 1995a; Berdoy, Webster and Macdonald, 1995). Thus,

in a competitive context directed to individuals of both sexes, be it to advertise social dominance or to warn intruders about aggressive intentions, females would not be expected to urine mark more than males.

4.4.1. Aims.

The aim of this experiment was to assess whether there is a sex bias in urine marking and investigation. The test consisted of recording the urine marking behaviour and investigation (extent of marks, time spent on tile and visits) of clean tiles by males and females at a close range. Because urine marks were measured from the TV monitor as they were deposited, not only the identity of the marking rat could be recorded, but also the extent of urine marks deposited *on each visit.* Such detailed recording of marking behaviour has not been reported before. Close range monitoring allowed me to obtain real rates of marking (i.e. recording even marks deposited on top of old marks) for each sex. Thus, if rats tend to urine mark over old marks, this method might show large differences in the extent of urine marks deposited by males and females that traditional methods had not shown. By establishing which sex, if either, marks more this might suggest the role urine marking plays in social communication (to advertise occupancy, to warn intruders, to attract mates, etc.).

4.4.2. Methods.

The rats used for this experiment were the wild caught pairs of rats forming colonies 4, 6, and 7. Because a female had died in pen 5, the experiment was conducted in only three pens.

The experiment consisted of presenting the rats with a clean tile and monitoring their visits closely using a close circuit colour TV camera sensitive to dim light. The camera was placed above the zenith of the tile and pointing directly down towards it. To achieve a clearer recording, a 60 W red light was placed by the camera.

The tiles were those described earlier (section 2.6). The tile to be used was washed and scrubbed with liquid detergent and water and then left to dry for at least one day. The clean tile used as a stimulus for marking always occupied the same location, at the corner by the door wall which was farthest from the door (at the front of the pens, see fig. 2.1 and 2.2). On the morning after the test, the clean tile (now marked) was replaced by the original native tile until another clean tile was introduced in the evening.

Urine marks from each single visit were recorded directly from video tape. The tile occupied its natural size on the TV screen, and thus the same grid used for measuring urine marks on tiles could be used to measure them on the TV screen. Each spot of urine deposited per visit was highlighted on the TV screen by outlining the borders with a waterproof marker pen. Thus, it was possible to distinguish new marks as spots not highlighted. Because new marks took a couple of minutes before they blended with the other marks, this method allowed new urine marks to be seen even when deposited over fresh ones.

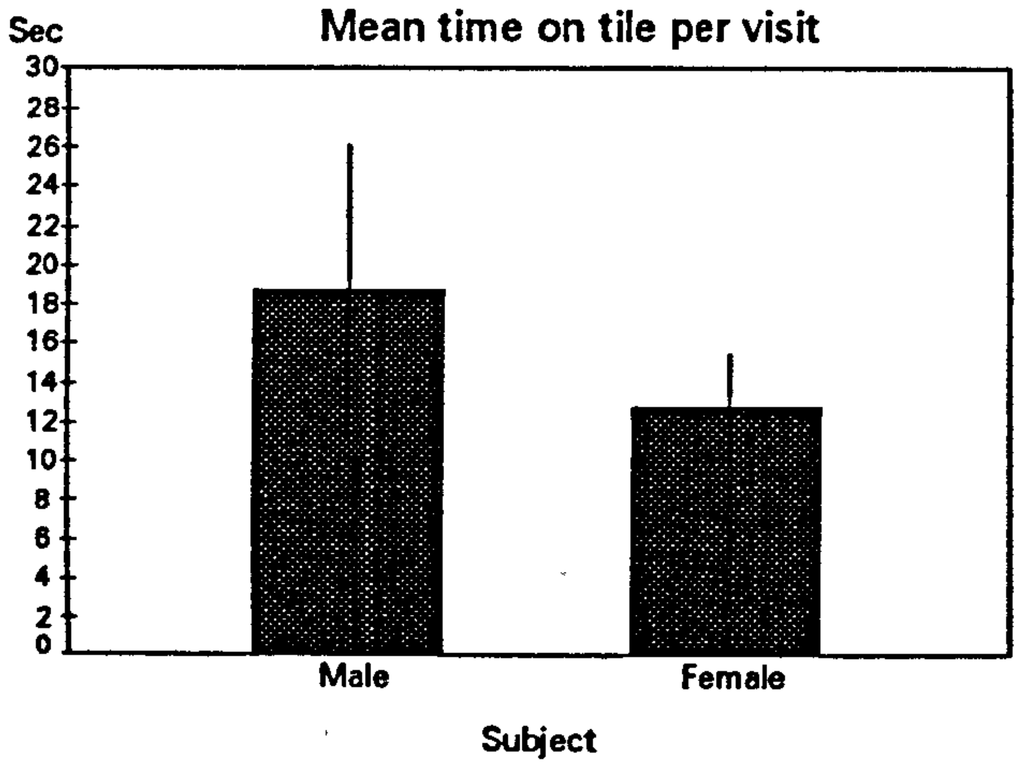
Trials lasted 8 hours (from 2100 to 0500 h, typically the active period of rats: Calhoun, 1962; Barnett, 1975; Nieder, 1985; and personal observation in these colonies). Five replicates were carried out on consecutive days in each pen. For each visit, the identity (sex) of the visiting rat, the time spent on the tile, whether it urine marked the tile and the extent of the marks were recorded. Visits

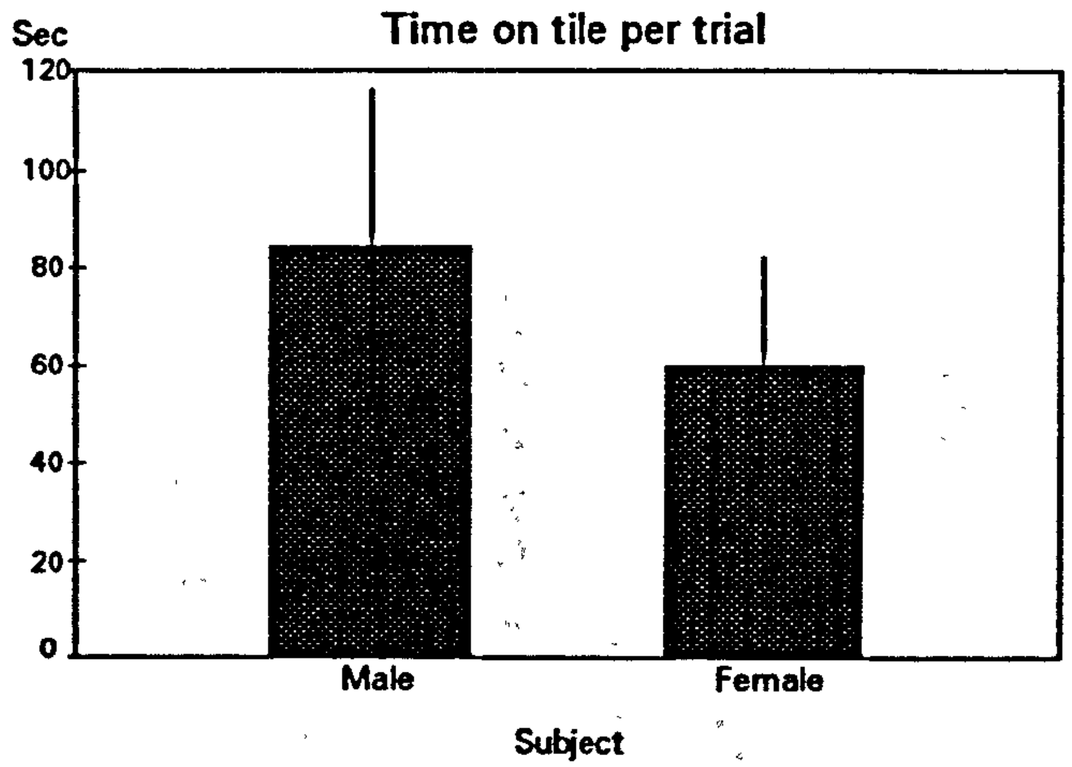
where the individual could not be identified were discarded. Although its percentage of the total was not quantified, data discarded for lack of identification constituted a minute proportion (less than 5%). During the transcription of the video, each visit was observed several times to extract the data reliably.

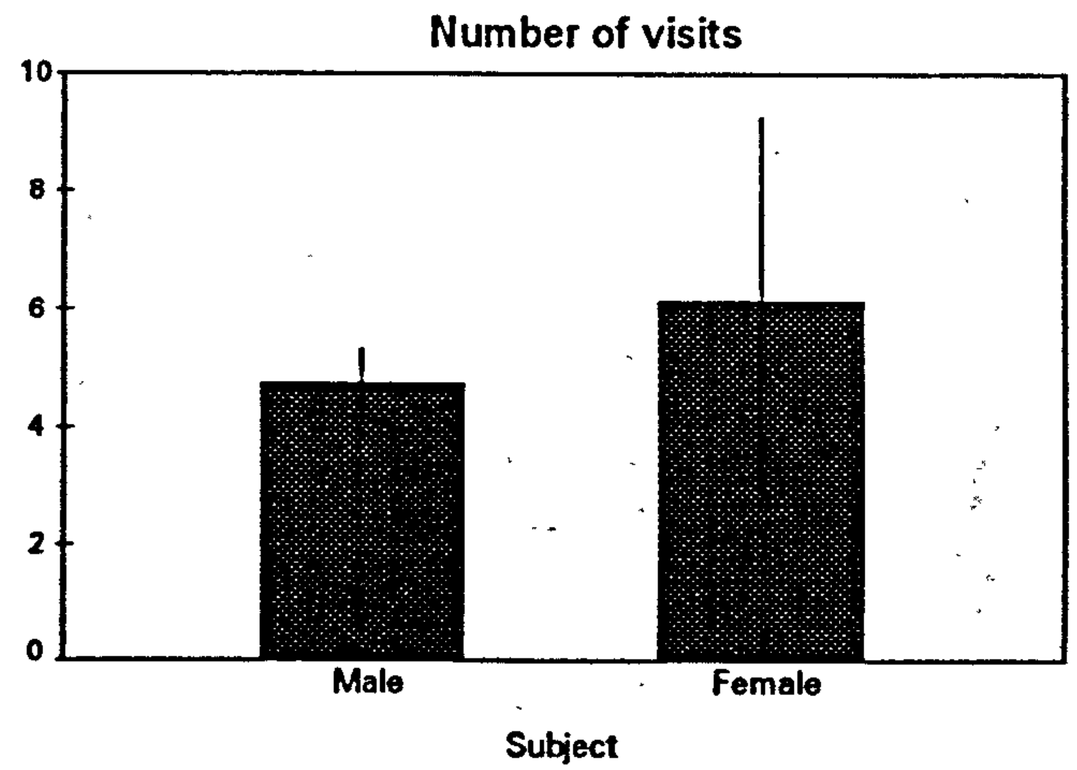
Data were analysed using non-parametric ANOVAs (Meddis, 1984). A one way non-parametric ANOVA tested the effect of day on each variable to assess whether rats habituated to the stimulus. A general test was used instead of a specific test because there were several possibilities for habituation: a gradual reduction over the five days, a large response on the first day or two and then a plateau, etc. Data were ranked for each individual independently (thus ranks had a value from 1 to 5) and individuals of both sexes were analysed together. To test whether male rats marked and investigated more than females, a specific-design two way non-parametric ANOVA was used. Data were ranked within each pen. Thus male and female ranks could be compared throughout the experiment and this also took into consideration the fact that measures within pens were non-independent. A consistent trend of higher marking and investigation by males was predicted. Thus the coefficients for the test were:

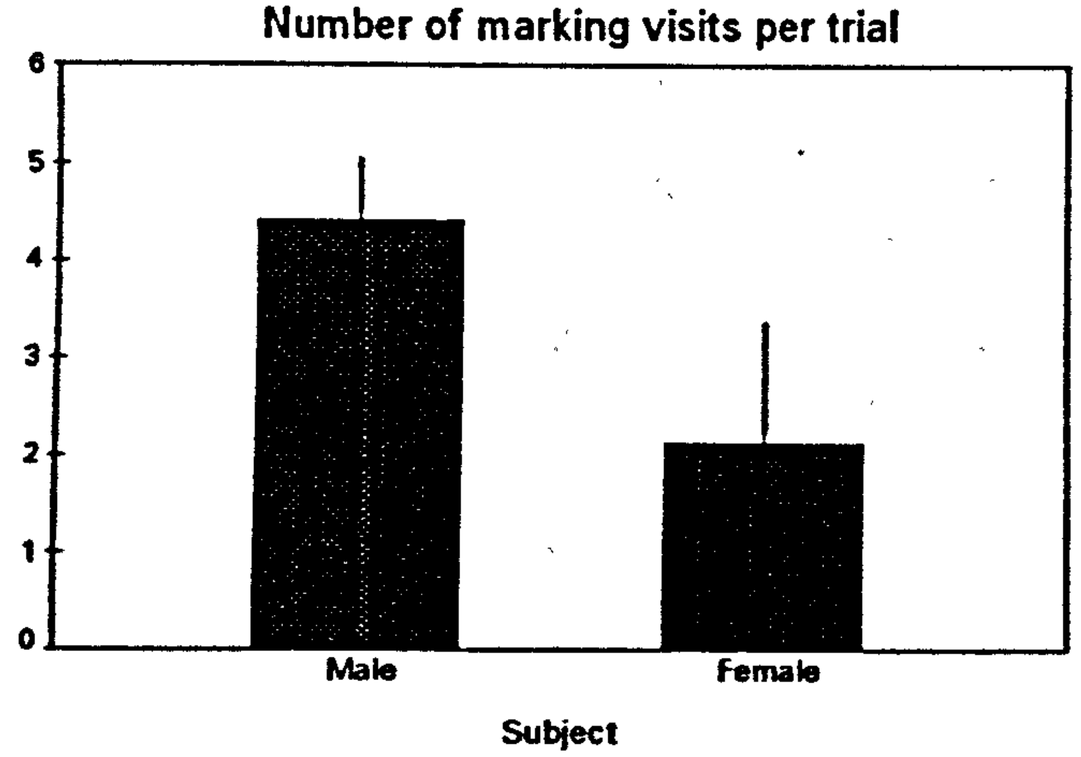
	day 1	day 2	day 3	day 4	day 5
males	+1	+1	+1	+1	+1
females	-1	-1	-1	-1	-1

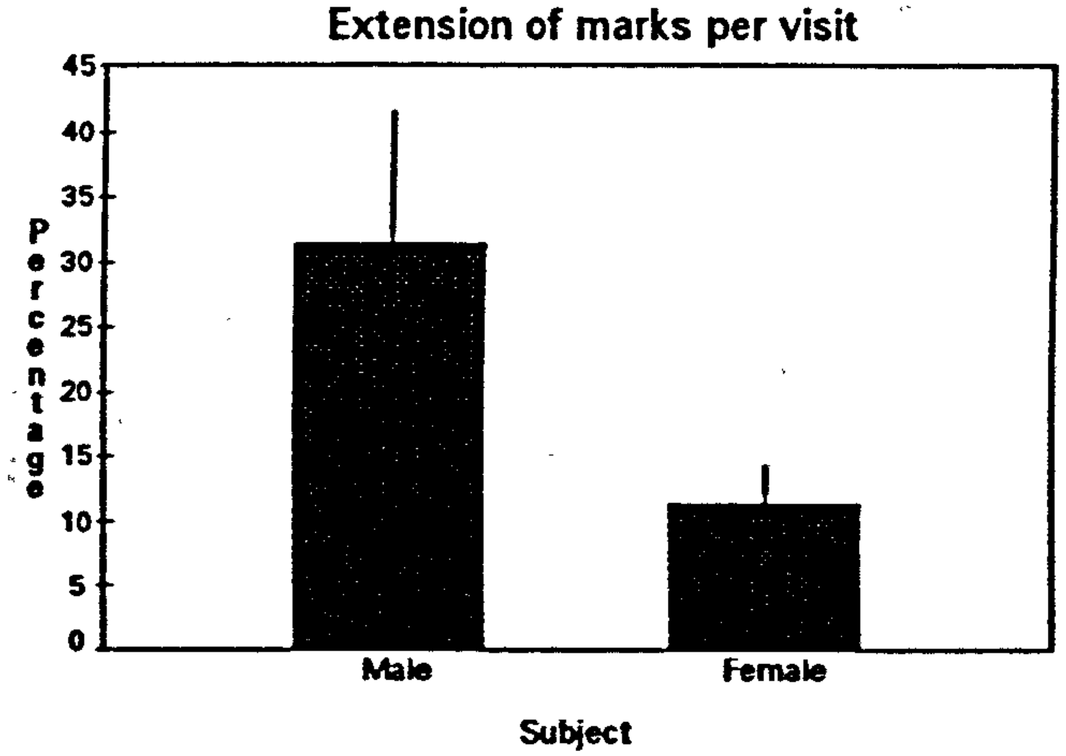
Because results showing the percentage of surface marked were computed from fresh marks which could be deposited over old











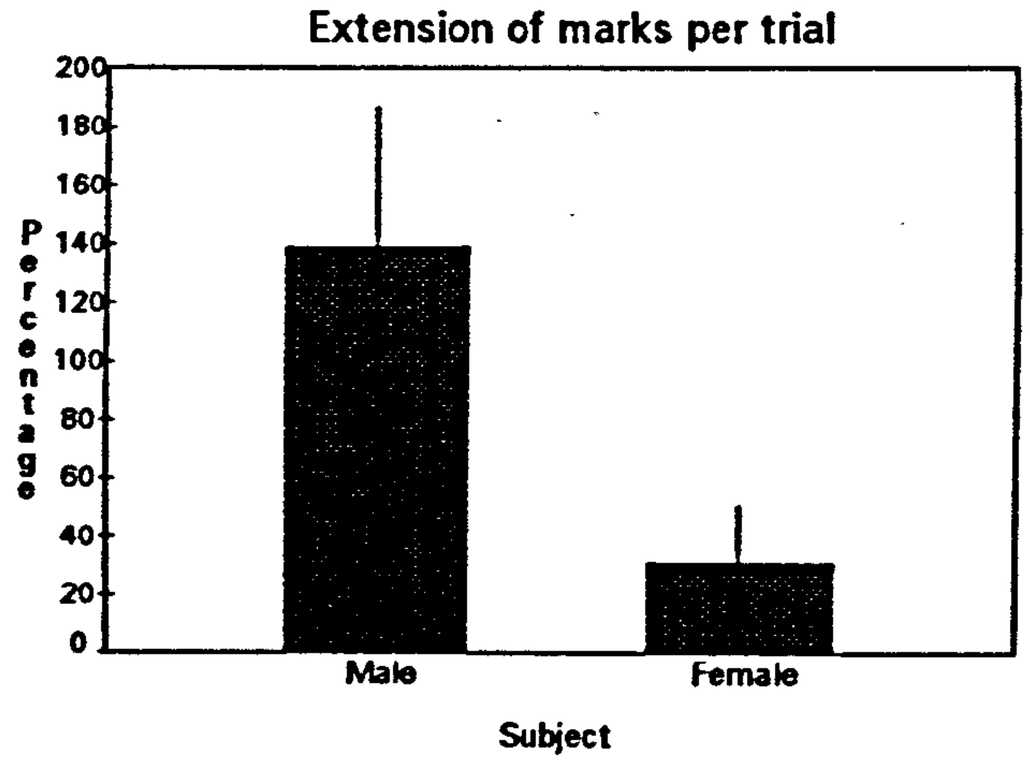


Fig. 4.8. Differences between males and females in urine marking clean tiles. Males marked in a greater proportion of visits and the extent of their marks both per visit (those in which individuals marked) and per trial, was larger than those of females. Males also spent more time per visit on screen than females, but, they did not spent more time per trial or visited the tile more often than females. Extension of marks measured as a percentage of the total surface of tile. Because marks were detected upon deposition and recorded even when deposited over old marks, final extension of male marks was greater than tile surface.

marks, mean percentages of more than 100% of tile surface covered by marks could be obtained.

4.4.3. Results.

No habituation or any difference between days was found for any of the variables analysed (extent of marks per visit in which marking occurred, $H_{5df}=7.40$, ns; extent of marks per trial, $H_{5df}=7.80$, ns; number of visits in which marking occurred, $H_{5df}=4.76$, ns; number of visits, $H_{5df}=3.95$, ns; mean time on tile per visit, $H_{5df}=0.73$, ns; total time on screen per trial, $H_{5df}=5.42$, ns).

Male rats marked more than females, both with respect to the extent of marks per visit in which marking occurred ($Z=3.95$, $p<0.001$; mean percentage ± SE of the tile surface covered by marks: males, 31.38 ± 10.06 %, n=3; females, 11.26 ± 3.00 %, n=3) and the total extent of marks per trial ($Z=3.93$, $p<0.001$; males, 138.60 ± 48.06 %, n=3; females, 30.74 ± 19.37 %, n=3). Data are presented in fig. 4.8. Males also urine marked on a greater proportion of visits than females ($Z=2.98$, $p<0.01$; mean proportion of marking visits to total number of visits ± SE: males, 92.45 ± 3.78 %, n=3; females, 62.97 ± 28.57 %, n=3). Females visited more frequently than males ($Z=-0.34$, $p>0.05$; mean number of visits ± SE: males, 4.73 ± 0.57 visits, n=3; females, 6.13 ± 3.13 visits, n=3). Females in two of the three colonies in this experiment seemed to make repeated attempts to escape. In many instances, the females stood in upright posture trying to climb up the walls (similar to the observations made by Hurst et al., 1996). This may explain the higher number of visits to the clean tile by females.

Although males spent more time on the introduced tile per visit than females did ($Z=2.03$, $p<0.05$; mean ± SE: males, 18.70 ± 7.37 s, n=3; females, 12.75 ± 2.69 s, n=3), the higher frequency of visits made by females resulted in a similar amount of time spent by each sex on the tile in total ($Z=1.54$, $p<0.05$; mean ± SE: males, 84.27 ± 32.25 s, n=3; females, 59.87 ± 22.24 s, n=3).

4.4.4. Discussion.

The results indicate that wild rats show a sex bias in urine marking. Males marked more than females both per visit and per trial. The results are consistent with those reported in the literature (section 4.4.1). As in the reports by Price (1977) and Brown (1991), males marked a larger extent of the tile surface than females. In my study, females visited the tile more often than males. This may explain why those reports in the literature looking at frequency of marking reported a greater marking by females (Lee, Mitchell and Adams (1984) and Peden and Timberlake (1990), for urine marking at the burrow entrance), in apparent contradiction with Price and Brown's studies. Lee, Mitchell and Adams (1984) and Peden and Timberlake (1990) recorded marking as the contact of the rats' genitals with the substratum, which is probably more frequent in females due to their greater number of visits but which does not always involve actual urine marking. However, a conclusive explanation cannot be offered because I could not measure contact of each rat's genitals with the substratum as a result of the overhead position of the camera.

Close monitoring of urine marking revealed that, although females visited clean tiles more often, the proportion of visits in which they actually deposited visible urine was much smaller than

males and, in fact, a greater proportion of visits by males resulted in visible urine marks. This method allowed me to detect even small drops of urine (the size of the small character in this print). It is thus unlikely that females, in contrast to males, were depositing marks which were too small to see. By discerning both the occurrence of urine marking and the extent of marks per visit, this experiment has shown a sex bias in urine marking in clearer detail than previously reported.

The greater extent of male marks suggests that urine plays a role in social communication, although, as pointed out before, the results could also be obtained if: i) males had a much larger bladder than females; ii) females had finer olfactory capabilities, or iii) males had a higher metabolic rate. These alternatives, however, do not exclude the communication hypothesis.

If males mark more than females because males are bigger and therefore have a bigger bladder and urine production rate, urine marking should be roughly proportional to body size. In one of the colonies there was difference of only 13 g between the male and the female (weight of the male 343 g, weight of the female 330 g) but the sex differences in their marking were still very high (mean ± SE; percentage of tile area marked per visit: male, 6.39 ± 0.86 %, n=5; female, 1.91 ± 0.33 %, n=5; percentage of tile area marked per trial: male, 12.20 ± 5.01 %, n=5; female, 3.96 ± 1.75, n=5). A 3% difference in body weight between the sexes is extremely unlikely to account for a 300% difference in the extent of marks deposited. In addition, this hypothesis would not explain why males investigated the urine marks deposited on the tile more than females did.

A second alternative might be that males have a higher metabolic rate than females, producing more urine without the

necessity of having a larger bladder. Although not unlikely, it would require a considerable difference in metabolism to explain the three fold difference in urine marking for a male and a female of similar size. It would presumably result in males having a far higher level of activity than that of females. This seems to be contradicted by the fact that females visited the tile more frequently than males and, therefore, seemed to have a higher rate of activity than males. Other researchers have also found that females showed activity rates greater than those of males (Gray and Lalljee, 1974; Lee, Mitchell and Adams, 1984; Hurst et al., 1996).

Although physiological/body size differences would result in greater urine marking rates by males, the existence of these does not exclude the possibility that urine plays a role in communication. This is because an explanation of the proximate causes can neither exclude nor validate a functional explanation (Sherman, 1988). Drickamer (1995) has found that urine production rates vary according to sex (males producing more than females) and social rank (the top male producing more than subordinates). He found that the greater rate of urine production in some individuals was consistent with their need to advertise. Thus, receptive females have a greater rate of urine production and they also urine mark more often (Drickamer, 1995), and the top ranking male produces more urine than subordinates, which is consistent with its greater urine marking rate (Hurst, 1990a).

Females may mark less than males because they have finer olfactory capabilities. Hence they would not need to deposit so much urine for use in orientation. However, no such difference in sensitivity is reported in the literature and it was not evident from my experiments. Moreover, superior olfactory capabilities in females

would be difficult to explain in terms of fitness advantages. It seems unlikely that males could not bear the evolutionary costs of having similar olfactory capabilities to females.

The greater marking rate and the greater length of time spent by males investigating both the tile and the urine marks deposited on it seems to be consistent with the hypothesis of competitive advertisement (Brown, 1970), although it could also serve to attract possible mates, as females do when they increase marking at oestrus (section 1.4.7.2.6).

As explained before, usually the top ranking individual in a colony is a male, and females are more often attacked by males than vice versa (section 1.4.5.1 and 4.4.1). Therefore, the defence of the territory from any intruder is more likely to be conducted by males than by females. As indicated by Brown (1970), territoriality involves not only aggressive behaviours towards intruders, but also advertisement of status/occupancy (range marking). The greater marking rate by males might be a means to advertise to intruder competitors than an area is occupied. Although male marking may also serve to attract mates, its main role seems more likely to warn competitors because these constitute a greater proportion of potential intruders (all intruder males plus all intruder females which are not receptive). This suggestion is consistent with reports in the literature because, whenever wild rats are tested in their familiar home range, males appear to regard intruder females as competitors, not potential mates (Barnett, 1958, 1975; Barnett et al., 1979). However, the results are not conclusive and further tests are needed to assess the urine marking and investigation response towards urine marks from intruders of both sexes.

Males might have investigated the clean and urine stimuli as a result of their novelty rather than to ensure that their marks predominate in their territory. However it would be difficult to explain why females were not so interested in such novelty. Males might have been specifically interested in familiar female urine on the test tiles for sexual reasons, and marking and investigating in response to this. However, the results show that the quantity of urine deposited by females was minimal. In addition, the most recent scents may mask previous scents, at least in golden hamsters (Johnston, Chiang and Tung, 1994), and thus, female marks are likely to be masked by male ones. Therefore, it seems more likely that males were scanning the substratum to ensure that their marks predominated over any others and that the density of urine marks matched that of surrounding areas (as the experiment in section 4.3 showed). In this case, we could predict that a high rate of urine marking by other members of the colony or unfamiliar rats would constitute a challenge, as has been found in mice (Hurst, 1993).

If, as mentioned before, the great difference in the extent of urine marks cannot be explained in terms of physiological differences, the question arises of where females deposit most of the urine they produce? It is possible that rats differentiate between urine excreted as waste and that used for marking. Perhaps males use most of the urine they produce in urine marking, whereas females use a small proportion and the rest is voided at certain sites in their home range (e.g. their resting chamber, somewhere in the burrows, etc.). It is also possible that sex differences in urine marking are only found when rats mark clean substratums, while are not apparent when marking familiar ones. Although it seems unlikely, it is nevertheless plausible and should be tested. However,

the problem with testing such hypothesis is that rats do not seem to mark very often on familiar marked tiles of the substratum and many replicates over a long period of time are likely to be needed.

4.5. Urine cues at food bowls.

. To assess the role that urine may play in social communication, a series of tests was needed that studied the responses of rats towards urine from different individuals. Some of these might, in principle, affect feeding behaviour, and thus a series of tests were designed to assess the effect of urine marks on the amount of food eaten.

Scents seem to be important in rat feeding behaviour. Rats can induce conspecifics to eat the diet they have previously eaten (Galef, 1990a). This process, known as social transmission of diet preferences, involves olfaction because anosmic rats do not show any preference (Galef, 1988). Some of the scents triggering this phenomenon are the odour cues left at food bowls like urine, faeces and foot gland secretions, although none of these sources seems to be effective separately (Laland and Plotkin, 1991). There appears to be a link between the urine trails rats produce (Calhoun, 1962; Telle, 1966; Boice, 1977) and social transmission of cues regarding food. Galef and Buckley (1996), have found that scent trails left by rats that have recently fed attract conspecifics. Although Galef and Buckley (1996) did not attempt to prove that the trails they studied involved urine, they might be similar to those reported by Calhoun (1962), Telle (1966) and Boice (1977).

Another important phenomenon related to feeding is that of neophobia. Rats, particularly wild individuals, are extremely reluctant to eat new foods (reviewed by Domjan, 1977). Neophobia

to new food containers is actually stronger than food neophobia (Shepherd and Inglis, 1987). If rat scent cues left at and around feeders can attract conspecifics and induce diet selection, the lack of those scents might be one of the factors producing neophobia.

Finally, scents may mediate competition for food. Although no overt aggression between rats has been observed at communal food hoppers, dominant individuals seem to exclude or deter other members of their group (section 1.4.5.1). Urine or other types of scent might play a role in such exclusion. E.g., urine from the dominant individual might be especially abundant at some food source to let other rats know where the dominant individual prefers to eat.

4.5.1. Aims.

The aim of this experiment was to investigate the role that urine marking and the olfactory background plays at food sources, and to assess the response of rats towards urine stimuli from individuals of different social rank and sex (dominant male, dominant female, subordinate male, etc.) at food sources.

4.5.2. Methods.

The experiment consisted of a choice test between two food bowls, one of which had urine applied to it.

Set-up. Each pen had two electronic balances on which the food bowls were placed. The balances fed the changes in weight to a computer, which calculated the food taken and weight of the individual eating. The system is fully described in section 2.6. Two infrared cameras recorded the images of both balances continuously to identify the individuals and timing of their visits. The cameras

were connected to a four-way splitter and to a video recorder, which allowed both images to be monitored at the same time on one tape.

Procedure. The test was conducted in colony 1 (containing 17 rats) and in colonies 4, 5, 6 and 7 (containing pairs of adult rats). The procedure was slightly different in both cases and only some of the treatments conducted in the large colony were repeated using the pairs of rats.

Procedure in colony 1. The food bowls were attached to a 32 x 32 cm base of aluminium sheet which allowed exchange of both the food bowls and surrounding background. The base had a metal cylinder screwed about 4 cm from the food bowl aligned along the diagonal of the sheet. Twenty two food bowls were placed in the pen: two with food in them placed on the balances and the rest sited between the balances and the front wall to serve as familiar stimuli with scent cues from the pen, although without food related cues.

Manipulations consisted of adding urine from a particular subject on top of the cylinder by one of the two feeding bowls. The urine had been collected when rats were in individual cages prior to their release. The control consisted of adding simultaneously a drop of water to the cylinder by the other feeding bowl. Sometimes the cylinder was unscrewed and exchanged with another one from a different location. Other manipulations consisted of swapping the whole bowl and base on one of the balances. The control in this case consisted of lifting the familiar feeding bowl on the other balance and placing it back in its original position. The following treatments were carried out with an appropriate control:

1. Addition of one drop of urine from the resident dominant male on top of the cylinder.

2. Addition of one drop of urine from a resident subordinate male.

3. Exchange of one feeding bowl with a familiar but unused food bowl.

4. Exchange one feeding bowl with a clean bowl.

5. Exchange of both bowls, one with a clean bowl and the other with a clean bowl painted with dominant male urine.

6. Exchanging the two feeding bowls with each other.

7. Exchanging the cylinder of one feeding bowl with a cylinder from a tile in the centre of the enclosure.

The aim this experiment was to establish whether scents deposited at non-feeding points reduced neophobia, particularly urine from the dominant male, and whether the orientation of marks had any effect on bait intake. Specifically, the aim of treatments 1 and 2 was to assess the role of social status on avoidance or attraction to food sources. Treatment 3 to 7 assessed the effect of scents deposited at food bowls, those deposited elsewhere, lack of scents from any rats, or the presence of scents from the dominant male, had on bait intake.

Trials lasted one hour. Three trials were performed every night with 14 replicates per treatment. The trial series started about 2100 h and finished about 0100 h. The video recorder was switched on when the first individual was observed to be out of the nest and stopped one hour later. After the trial ended either the feeding bowls were placed back in their original positions or the top of the cylinder was cleaned with a cotton swab soaked in 96° ethyl alcohol. The order of the treatments was randomised by day and order during the session (first, second or third treatment in a session). Only treatments 4 and 5 were relegated to the end due to

the expected disturbing effect of introducing a neophobic stimulus. The bowl to be treated, i.e. left or right, was alternated from trial to trial of a session, and sessions started on alternate sides on consecutive days.

Data from video tapes (frequency and timing of visits and identity of visiting rat) were stored on a computer spreadsheet for subsequent analysis. Data from balances were computed to obtain number of feeding bouts and measures of food taken per trial (mean per bout and total per trial).

The results from video tapes were first tested to assess the rats' preference for left/right side across treatments (which could arise, for example, because rats avoided the food bowl closest to the pen door). Then, results were tested for habituation and for differences in activity due to the trial order in the session using a two way ANOVA.

Procedure in colonies containing pairs of rats. Food bowls and aluminium sheets were as those used in colony 1, but the metal cylinder was removed. Only the food bowls placed on the balances were used. In contrast with the procedure in colony 1, urine was applied all around the edge of food bowls, so that the rats found it whichever direction they approached the bowl from. Controls consisted of application of water to the edge of the control feeding bowl. In treatments involving replacement of one bowl with a clean bowl the control consisted of lifting and placing back the other feeding bowl. The experiment consisted of the following treatments:

1. Test of preference between left and right food bowl.
2. Addition of resident male urine.
3. Addition of resident female urine.
4. Addition of neighbour male urine.

5. Addition of unfamiliar male urine.

6. Addition of resident male urine on one bowl versus neighbour male urine on the other.

7. Addition of neighbour versus unfamiliar male urine.

8. Exchange one bowl with a clean bowl.

9. Exchange both bowls, one with a clean bowl and the other with a clean bowl painted with resident male urine.

The aims were similar to those of the previous experiment. However, because the composition of the colonies was different, so were some of the treatments. Thus, treatments 2 and 3 assessed the joint effect of sex and status of the resident on the response to food, whereas treatments 4 to 7 (and compared with treatment 2) assessed the effect of the familiarity of the urine donor.

As a female died in colony 5, only three pens contained a pair of rats. Each treatment consisted of six replicates. As in the previous colony, a replicate lasted one hour from the moment the first rat came out of the nest. The type of treatments that were carried out in each day's session and their order was overall randomised as explained earlier, but treatments involving clean bowls were relegated to the last trial of the night. As before, the bowl (left or right) where the experimental stimulus was to be applied alternated from treatment to treatment, and from day to day.

After the trial finished, the edge in both bowls was cleaned with a cotton swab soaked with 96° alcohol.

The activity of the rats was recorded as before, but each camera recorded both feeding bowls at the same time.

Data were analysed using multi-variate analysis of variance, MANOVAs (repeated measures factorial design) on the time spent on the balances and the frequency of visits.

4.5.3. Results and discussion.

In colony 1, rats did not habituate to the stimuli over the experimental period, nor was there an effect of order in a day's session (first, second or third treatment in a night). Thus sessions of 3 trials per night were also conducted in the pair colonies.

Most of the tests failed to reveal a statistically significant difference in time spent on one of the bowls within a treatment. In subsequent analysis the effect of treatments was found to be either undetectable or very weak. The treatments in colony 1 analysed with ANOVAs failed to reach statistical significance. So did the treatments carried out in colonies 4 to 7, analysed with MANOVAs. There was a weak difference in time spent on one of the bowls during the first 4 visits, which changed for the other bowl after them, but this was a post-hoc analysis, and I did not predict that rats would change their response after the first visits. Furthermore, the change in response was achieved after the fourth visit in some of the treatments, but not in all of them. Only those treatments involving clean bowls produced a significant response over the whole trial, stimulating avoidance from the rats.

It was uncertain whether the general lack of response was due to the weakness of the stimuli, the length of the trial or some uncontrolled factor. For this reason, the stimulus was applied onto the edge of the bowl instead of onto the top of the cylinder in colonies 4 to 7. However, the response continued to be weak. There was also the possibility that rats respond differently to urine

cues applied by a researcher and those applied by the rats themselves. Such a difference was found for faecal marking in response to urine cues from rats belonging to other colonies (sections 3.4, 3.5 and 3.6). In addition, Birke and Sadler (1984) also found a significant response towards a urine stimulus only if the marks had been deposited by the rats and not towards those applied by the experimenter. Alternatively, the composition of urine may have been different in urine deposited for communication purposes compared to urine collected from individually caged rats.

4.6. Pilot experiments on olfactory manipulations using tiles.

4.6.1. Aims.

Olfactory manipulations similar to those described in the previous section for colony 1 were carried out using tiles. Experiments were also conducted using urine stimuli from rats of different social classes. The aims were to find evidence suggesting the role that urine plays in orientation and social communication (e.g., whether rats would be able to detect manipulations in the olfactory background, whether they were attracted to urine from some individuals more than urine from others, etc.). The set of experiments was designed as a group of pilot experiments to suggest future experimental lines to be developed within the present project.

4.6.2. Methods.

Set-up. Fifteen quarry tiles were placed in the central area of the pen. The tiles occupied three rows each with five tiles. Each tile was equidistant from its neighbours (at approximately 50 cm from them), and the set was placed between the nesting and feeding

areas. Between the tiles and the walls, nest, and food bowls there was a distance of at least one metre. The tiles had a cylinder screwed in their centre as described in section 2.6.

A set of four tiles had been placed along one of the side walls to compare the density of urine marks with those in the centre. A tile had also been placed at each of the four corners of the enclosure. Two cylinders had been glued by their screw to the drainage channel, which was frequently used by rats, to compare responses to highly marked cylinders with others infrequently marked (those on the tiles in the centre).

Activity was recorded using two cameras, one for the experimental tile and one for the control. The cameras articulated through two axes which allowed them to reach any position in a horizontal plane. One of the axes was a Dexian frame transverse to the longer axis of the rat pen and held at a height of about 1 m by two Dexian arms bolted to the ceiling. The other axis of the cameras rotated horizontally in the first one through a bolt. A counterbalance weight at the opposite end of this axis held the cameras in the horizontal plane. The image of these cameras was fed to the same tapes as the images from feeding points described for the large colony.

Procedure. The trials again lasted one hour and were conducted simultaneously with trials at feeding bowls. Therefore, they had the same timing, number of replicates (14), treatments and treatment order as those on feeding bowls. Manipulations consisted of the following treatments:

1. Addition of urine from dominant male on top of the cylinder.
2. Addition of urine from a subordinate male.

areas. Between the tiles and the walls, nest, and food bowls there was a distance of at least one metre. The tiles had a cylinder screwed in their centre as described in section 2.6.

A set of four tiles had been placed along one of the side walls to compare the density of urine marks with those in the centre. A tile had also been placed at each of the four corners of the enclosure. Two cylinders had been glued by their screw to the drainage channel, which was frequently used by rats, to compare responses to highly marked cylinders with others infrequently marked (those on the tiles in the centre).

Activity was recorded using two cameras, one for the experimental tile and one for the control. The cameras articulated through two axes which allowed them to reach any position in a horizontal plane. One of the axes was a Dexian frame transverse to the longer axis of the rat pen and held at a height of about 1 m by two Dexian arms bolted to the ceiling. The other axis of the cameras rotated horizontally in the first one through a bolt. A counterbalance weight at the opposite end of this axis held the cameras in the horizontal plane. The image of these cameras was fed to the same tapes as the images from feeding points described for the large colony.

Procedure. The trials again lasted one hour and were conducted simultaneously with trials at feeding bowls. Therefore, they had the same timing, number of replicates (14), treatments and treatment order as those on feeding bowls. Manipulations consisted of the following treatments:

1. Addition of urine from dominant male on top of the cylinder.
2. Addition of urine from a subordinate male.

3. Exchange of a tile cylinder with one of the cylinders stuck in the drainage channel.

4. Exchange of a tile with a clean one.

5. Exchange of a tile with an experimental clean tile painted with dominant male urine, using a clean tile as the control.

6. Central tile swapped with a tile by the wall.

7. Exchange cylinder of a tile with one from a food bowl.

The manipulation procedure and aims were similar to those previously described for bowls. Water was applied on top of the cylinder or surface of the control tiles whenever urine was applied on experimental tiles. The top of cylinders were cleaned with cotton swabs soaked with alcohol after each trial and before the first trial of a series. To reduce confounding effects of distribution pattern, urine on clean tiles was applied thoroughly over the whole surface of the tile and not on the top of the cylinder. Urine marks were recorded with a 0.25 cm^2 grid. The location of each pair of tiles was selected at random excluding those already used in previous trials of the current session. The order of the trials was random and coupled with the same treatment conducted at feeding bowls. Trials involving neophobic stimuli were relegated to the end of the session.

4.6.3. Results and discussion.

The rats avoided all the tiles and deposited almost no marks on them (see section 4.2.1 for extent of urine marks on the tiles in the centre, by the wall and at corners). Thus, there was virtually no urine background to manipulate. No urine marks were deposited either on the experimental or control tiles during the trials. In contrast to the feeding bowls, rats did not visit the tiles very often. Most of the replicates did not receive a single visit. Therefore, most

of the cells for an ANOVA were empty and no analysis could be attempted. Not even the presentation of a clean tile for one hour triggered a response by the rats. Only painting a clean tile with dominant male urine occasionally attracted some rats. The failure of the experiment seemed to be due partly to the fact that rats were very reluctant to mark the tiles and that there was no urine background. One hour was probably not enough time to trigger a response. Another possible reason for the lack of response may be that the centre of the enclosure constituted the area of the pen least visited by rats. Rat activity and the chance to find a stimulus on a tile was greater by the walls, and thus, subsequent experiments were carried out using tiles by the walls of the pen. In contrast to reports by Brown (1975), Price (1975) and Hopp and Timberlake (1983), rats were not attracted to mark the cylinders, whereas they marked the plain tiles used in subsequent colonies abundantly.

4.7. Response to artificial urine marks from different donors presented on clean tiles.

4.7.1. Aims.

This experiment was similar to the experiment on faecal discrimination, and tested whether rats could discriminate among urine from different individuals. A significant response would imply that rats can use information encoded in urine. If urine was being used for some kind of social communication purpose, the urine marking response should differ according to the individual used as a donor.

4.7.2. Methods.

Rats were presented with a choice between a clean tile and a clean tile with a streak of urine. The experiment was carried out in colonies 4 to 7. Tiles were those described in section 2.6 without the cylinder. The urine was collected when rats were caged individually before their release in the pens. Urine was stored in 1 ml doses and frozen to prevent chemical or bacterial decay. The tiles were placed by a wall, alternating the experimental tile between two different locations on consecutive days and the control between another two. The tile closer to the hay stack was always the first visited by the rats. For this reason, experimental and control tiles alternated in the closer position to the hay stack on consecutive days. Experimental and control tiles were always placed in consecutive order without any native tile between them (i.e., they were 0.5 m apart).

An infrared camera attached to the shed ceiling monitored the activity of the rats at both tiles in the same picture. A 60 W red bulb hung above and between the tiles at about 1 m to increase visibility of the image. The picture from two pens at the same time was fed to a four way splitter and a time lapse video. The tapes recorded the images from this experiment and that on faecal discrimination (section 3.4) at both enclosures. As the tiles were placed along the most frequently used pathway, the wall separating two pens, rats came across the tiles used in this experiment very frequently. Several variables were recorded for each visit, including the identity of the individual, the first and last time when it contacted the tile either with its snout or feet and whether there was snout contact with the tile.

Urine marks were recorded using a 0.75 cm^2 grid described in section 2.6. Marks were recorded the morning following the experiment, at about 1000 h. In contrast to the experiment on sex bias in urine marking (section 4.4) marks could not be ascribed to individuals or visits. It was not possible to detect when a rat was urine marking because the recording did not show sufficient detail, and records were taken from an angle, not exactly above the tile.

The experiment consisted of four treatments. The treatments were the same as those in the faecal discrimination experiment:

1. Streak of urine from the resident male.
2. Streak of urine from the resident female.
3. Streak of urine from the neighbour male.
4. Streak of urine from an unfamiliar male.

Each treatment was replicated nine times. A replicate was recorded for 8 h (2100 to 0500 h), but, as urine marks were recorded in the morning, marking was allowed for more than 12 h. However, visits after 0500 h were unlikely (section 3.3 and 3.4).

4.7.3. Results and discussion.

Although rats visited tiles very frequently every night, they showed no difference in response either between the urine from different donors or between the experimental and control tiles. The reason for this is unclear :

i) The clean surface may have constituted a stimulus stronger than the streak of urine. Rats are strongly attracted to clean tiles and they mark them abundantly (section 4.3). Thus, they may have missed the streak of urine during their marking visits. Additionally, rats may have either responded to or masked the urine stimuli with

their own marks during their first visits and subsequently responded to the still clean area of the tile.

ii) The amount of urine applied to the tile may not have been enough to attract the attention of the rats. Therefore, the response observed would have been only the response to the clean part of the tile.

iii) The urine applied to the clean tile may have been qualitatively different to that rats use for communication purposes. Thus, rats may have detected the urine, but they failed to respond to it because it was an uninteresting type of urine. As Waldman, Frumhoff and Sherman (1988) pointed out, at the behavioural level it is only possible to detect scent discrimination (a difference in response). Animals may recognise scents (the neural process underlying discrimination), but they may not have the motivation to show a response. Chemicals used for communication may trigger that motivation in marks deposited by the rats.

iv) The continuous movement of the rats over the tiles (sited along a main path) may have added a great amount of extra data when rats were not responding to the tiles, rendering the statistical analysis non-significant.

v) Some other reason or uncontrolled factor may have influenced the response of rats. The order in which rats found the first tile of the test was found to be a significant factor, and thus, it was taken into consideration when analysing the data. However perhaps this effect, along with the high variability, both between days and between colonies, may have obscured the response of the rats. This would only happen if the response of the rats towards the stimuli was small, because the same sample size showed a significant response in faecal discrimination (section 3.4).

4.8. Urine counter-marking in response to urine from non-residents.

4.8.1. Aims.

The experiment in section 4.4 and evidence from the literature suggested that urine may play a role in social communication. However, the experiments in sections 4.5, 4.6 and 4.7 showed that rats failed to respond to urine applied by the experimenter. If rats use urine to communicate with other rats they should respond to marks deposited by other rats, and the response to marks deposited by other individuals should be stronger than to their own. An experiment was carried out to assess the urine marking in response to urine marks naturally deposited by rats from other colonies compared to those deposited by the resident pair. The experiment used cues from both males and females on the same tile because it was considered important that the marks were deposited by wild rats in their home pen. Wild rats do not habituate well to cages and the previous experiment suggested that their response may change if marks were deposited by the rats themselves and also if they were deposited while in their home pen.

Because the stimuli were deposited by a pair of rats in response to a clean tile and, therefore, most of the urine deposited belonged to males (section 4.3), the test was probably assessing the response of rats to urine from resident male compared to that from alien males. This may add further evidence to the role that urine plays in communication between competitors.

4.8.2. Methods.

Rats were presented with a choice test between two tiles, one naturally urine marked by residents and the other by rats from other colonies. Because colony 5 contained only a male and also because this rat urine marked only scarcely, the test was conducted in colonies 4, 6 and 7. A pair of wild adult rats, one male and one female, was housed in each pen.

Each trial consisted of two parts: a session to collect urine marks and the trial itself.

Two clean tiles were placed inside each enclosure to allow rats to mark them overnight. One of these tiles was destined to be used as a control during a trial on the following night while the other was exchanged with a similarly-treated tile from the neighbour pen. This latter tile was the experimental tile. Thus, it was possible to compare the response of the residents to similarly marked kinds of tiles: those tiles marked by rats from other pens (non-resident rats), and tiles marked by residents as a control.

Before and after each trial was finished, the marked surface of each tile was measured using the 0.75 cm^2 grid described in section 2.6 to compare the urine counter-marking response of resident. Once the trial ended, both tiles were washed with water, liquid detergent and a scourer. They were rinsed and left to dry for at least one day before being re-used. Controls were assigned to one pen and never exchanged with another so that any remnants of alien urine marks from past trials could not possibly interfere with the current residents' marks. Because experimental tiles always ended up with a mixture of marks from own and other colonies, these tiles were not re-assigned to a pen or utilised as a control even after cleaning. Trials were set up just before the onset of the rat's

activity period, at about 2100 h. During the session to collect marks, tiles were left continuously in the pen until 2100 h the following night. All tiles used were marked. Urine marks on the tile were then recorded, one tile of each pair was exchanged with a similar tile from the neighbour enclosure and the trial started. Marks were again measured the following morning at about 1000 h. Each trial, thus, lasted more than 12 hours (although the activity period of rats usually ended at 0500 h). The test was repeated nine times in each enclosure. Clean tiles to be used as stimuli for the neighbour pen were placed in pen 5, but the test itself was not conducted on this male. No video record was taken in any pen. The only variable measured was the extent of the marks before and after the trial.

The extent of the marks was expressed as a percentage of the tile surface. Because both experimental and control tiles collected urine marks using the same method, the data used were the overall extent of marks after the experiment (old and new) and not the estimate of counter-marks on both previously clean areas and over urine marks (the *real rate of marking*, section 4.3.2). The results from the response to urine marks from own or other colonies were matched for each pen. Data were analysed using two-way non-parametric ANOVAs (Meddis, 1984) testing for the effect of urine donor and colony. The first test consisted of a comparison between the extent of the counter-marks on the tile urine marked by residents and that marked by neighbours. Counter-marking on urine marks belonging to neighbour rats was expected to be greater than marking on the colony's own marks. Thus, the coefficients for this specific test were:

counter-marking on	pen 4	pen 6	pen 7
neighbour cues	+1	+1	+1
resident cues	-1	-1	-1

However, rats may deposit more marks on tiles marked by neighbours because, incidentally, there happened to be more urine on a neighbours' tile than on those marked by residents. Although unlikely, to prove that this was not the case, a second test analysed the overnight marking in terms of the surface marked before the trial (e.g. if before the trial urine marks occupied 1 square unit and after it they occupied 2 square units a 100% of increase in the extent of urine marks was computed). Thus, a doubling in the surface of urine marks was regarded as the same increase regardless of the absolute value of the extent of the stimuli marks. In other words, the same area covered by urine after the trial was considered as a stronger response on tiles with fewer stimuli marks than on tiles with many stimuli marks.

The coefficients for the test were as before.

4.8.3. Results.

Both predictions were confirmed. Rats counter-marked tiles marked by neighbours more than those marked by residents. Tiles previously marked by rats belonging to other colonies showed a greater increase in the percentage of tile covered by urine marks after the trial than tiles marked by the residents themselves ($Z = 5.12$, $p<0.001$; mean percentage of increase on tile surface marked $\pm$ S. E.: tiles with marks from non-resident rats, 12.8 ± 4.4 %, $n = 3$; tiles with marks from resident rats, 3.2 ± 1.81 %, $n = 3$, see fig.

4.9). This effect could not be attributed to a greater extent of stimulus marks on the tiles marked by rats from other colonies than on those marked by residents: counter-marking of cues from neighbours was greater than that of marks from residents even when variability in the area occupied by the stimuli was taken into account ($Z = 2.43$, $p<0.01$; see plate 4.2).

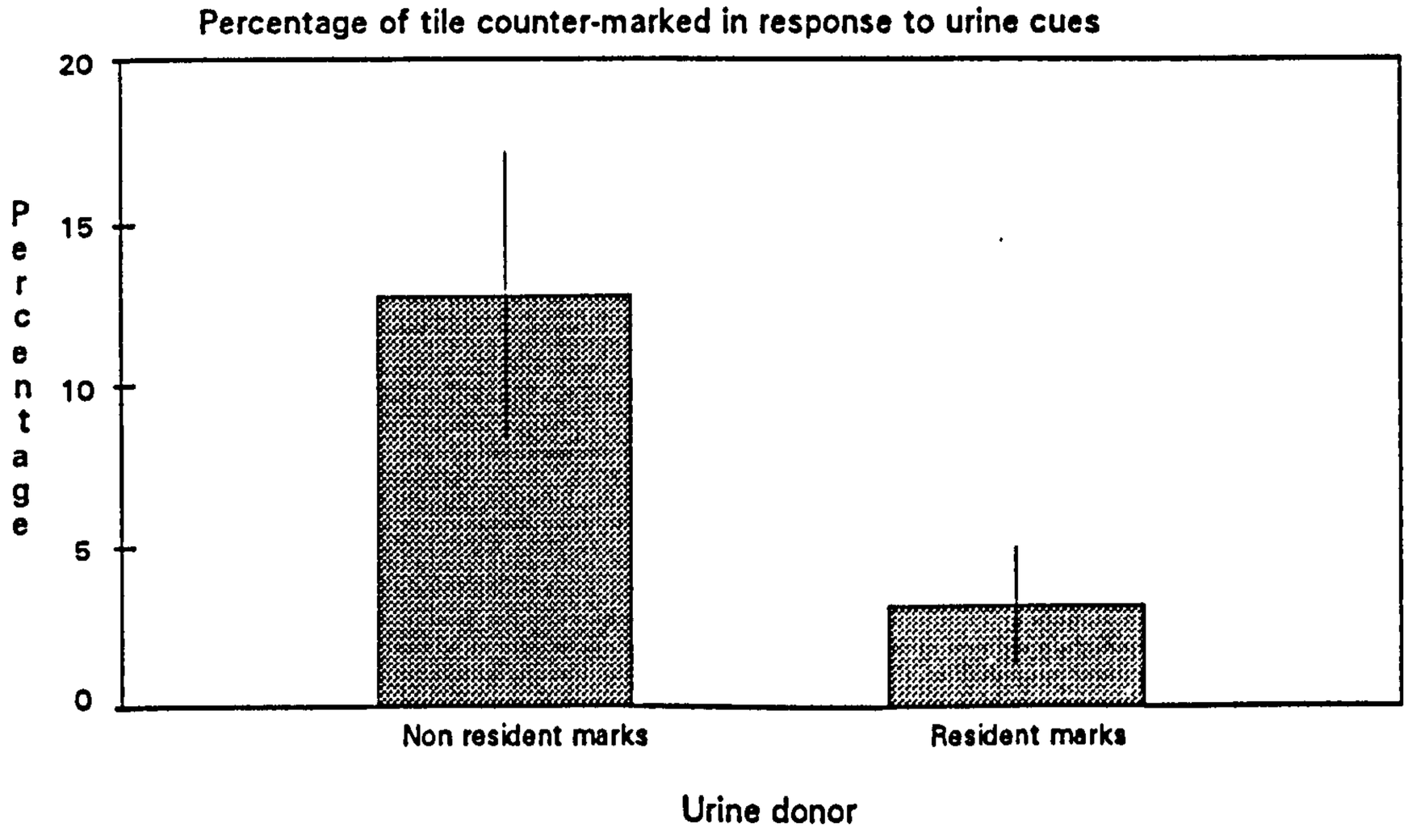


Fig. 4.9. The percentage (mean ± standard error) of tile surface urine counter-marked in response to existing urine marks from rats from either other or own colony.

4.8.4. Discussion.

Urine counter-marking in response to alien cues was greater than in response to the colony's own marks. This effect could not be attributed to a greater amount of marks serving as stimuli on the tiles bearing cues from neighbours because counter-marking towards non-resident stimuli was greater even when the area occupied by the stimuli was taken into consideration. Thus, it was the kind of donor and not the amount of marks serving as stimulus which triggered

Plate 4.2. Extent of marks on a previously clean tile after it had been marked overnight in a pen (upper photograph, first part of the test), and after a similarly marked tile had then been transferred to a neighbouring pen for a second night (bottom photograph, second part of the test). The dark areas indicate urine marks. The increase in surface covered by marks was remarkable, especially when the stimuli were marks from neighbour rats (bottom photograph). In this case it was common to find faeces around the tile (faecal marking). Both are representative of each stage of the test.

more marking. Rats seemed to be more interested in alien urine marks than their own. Similar results have been reported in the literature using laboratory rats in clean test cages, where conspecific (unfamiliar) odour cues elicited more marking and investigation than own cues (Brown, 1975, 1977, 1985, 1991).

The results show that rats could discriminate between their own marks and those of another colony. Although urine is presumably the cue, and it was the only chemical cue discernible by sight, it is not possible to rule out the possibility that other scent cues (secretion from the foot pads, from the sebaceous glands lubricating the fur, or some other type of cue) might be responsible for the response observed. Adams (1976) found that urine marking by an intruder triggered both urine marking and aggression in resident male laboratory rats. However, the scent cues left by the rats may belong to the preputial gland (which does not open to the urethra but onto the surface of the penis/clitoris (Brown and Williams, 1972). Products released from the preputial gland have been shown to be attractive (section 1.4.7.2.10). The secretions of the sebaceous glands have also been found to induce behavioural responses (Calhoun, 1962; Natynczuck, 1990; Peden and Timberlake, 1990). The experiments reported in sections 4.5, 4.6 and 4.7 could also have failed because the urine applied by the experimenter lacked the semiochemical used in communication. Alternatively, urine itself may not be used in communication but it may be the precursor of the biologically active compound (i.e., ageing might produce the active chemical). This seems to agree with Price (1977) who found that rats investigate male urine aged 7 to 8 days more than fresh urine. However, the response to aged urine may vary greatly depending on the donor and the context: Lydell

and Doty (1972) found that males investigate urine from oestrous females for longer when fresh than when aged 1 to 3 days. That is, the message announcing female receptivity is likely to be outdated when the urine is aged, whereas aged urine deposited widely on the substratum may indicate that several days have gone by without being counter-marked by another male and, therefore, the owner of the marks is likely to be also the owner of the territory.

Another possibility to explain the difference in response to natural and artificial marks may be that urine was not the scent producing the response in the present experiment (which would explain why applied urine failed to produce a response in experiments reported in sections 4.5 to 4.7).

Assuming that urine produced the response, these results suggest that it plays a role in social communication. Individuals responded to urine from other rat colonies more than towards their own. If urine played a role only in orientation, no difference in counter-marking should be expected towards two types of tile similarly urine marked and differing only in the type of the urine donor. Such was the case found by Hurst (1987) in mice, where marking by unfamiliar mice reduced the marking response towards clean acetate sheets. It might be argued that residents marked the tile with marks from rats belonging to other colonies more because they preferred to use their own urine cues for orientation. However, if urine does not play a role in social communication it would be difficult to explain why females marked clean tiles so little, and why males investigated urine marks from non-resident rats more than females did, and why resident males only faecal marked urine cues from non-residents and not their own.

As most of the urine marking is done by males (section 4.4), the greatest proportion of stimulus marks belonged to males. Because scent marks seem to mask previous marks (Johnston, Chiang and Tung, 1994; Johnston, Munver and Tung, 1995), the few female marks present in the stimuli may have been obliterated. In this case, it is likely that the response found was towards male cues. It could be argued that, perhaps in the case of marking stimulated by neighbour males, the female marked far more than the male, but that seems unlikely because faecal marking occurred at the same time as urine marking, and this behaviour was also conducted mainly by males. Greater marking by males towards male marks would imply a competitive role for urine marking. The experiment on faecal marking towards urine cues from rats belonging to other colonies (section 3.5) showed that males investigated the tiles bearing a mixture of urine from male and female non-resident rats more than females did. They also investigated urine marks from unfamiliar laboratory males more than females did. Both responses are consistent with the results showing greater counter-marking (presumably made by the resident male) towards a mixture of predominantly male urine.

However, the results are not conclusive. The tile presumably had female urine on it too and a response to that urine cannot be ruled out. Although it might seem that such marks ought to have been masked by the more abundant male marks, males might be especially sensitive to female marks. Additionally, the small amounts of urine deposited by members of the group other than the dominant male, although scarce, seem to be detectable in mice, and play a role in maintaining tolerance among the group members (Hurst, Fang and Barnard, 1993).

Laboratory rats tested in clean unfamiliar arenas have been found to be able to discriminate between their own urine, urine from other males and that from females (Brown, 1975, 1977, 1985, 1991; Birke and Sadler, 1983, 1984; Moore and Samonte, 1986). It could be argued that perhaps both types of tile were marked at a similar rate, but marks were especifically overlaid on top of old marks when these came from the residents themselves, so that these obscured the new fresh marks. However, this is unlikely because such counter-marking would be expected to be directed towards marks from other colonies, not towards own ones (sections 1.3.2.2.1 and 1.3.2.2.1.1). Thus it appears that marks from non-residents stimulated a considerably greater counter-marking response (twice greater) than the resident's own marks.

CHAPTER FIVE
GENERAL DISCUSSION

5.1. Faecal marking.

The results of the experiments reported in sections 3.4, 3.5 and 3.6 suggest that rats may use faeces in communication and that rats respond with faeces to scents from other rats.

The results from the experiment on faecal discrimination (section 3.4.3) show that rats can discriminate among different types of faeces. Such discrimination is presumably based on chemical cues. This, in turn, suggests that rats can use olfactory information carried by faeces, and that faeces might be used in communication.

Rats also used faeces to respond to conspecific urine marks. Faecal marking is not a response to non-social novelty because clean tiles, which triggered urine marking (which might constitute a response to non-social novelty), did not elicit faecal marking. For the same reason, faecal marking cannot be explained as incidental deposition correlated with the time that rats spend in different sites (section 3.5.5). Rats investigated the clean tiles for a long time but they never faecal marked them. Furthermore, they did not faecal mark tiles bearing resident urine marks. Faecal marking was not associated with urine marking. When both urine and faecal marking occurred together, rats deposited urine while they were on the tile, whereas they defecated when grooming themselves a few centimetres away from the tile bearing the stimulus.

These experiments extend Leon's (1974) finding that faeces from pregnant or lactating females attracted pups. However, this is the first suggestion that rats that are not pregnant or lactating use faeces in communication. Leon found that only caecotrophes (soft

and moist faeces that have not been re-digested) attract pups. In the faecal discrimination test reported in this thesis, the faeces used appeared to be a mixture of caecotrophe and common faeces, although distinction between them sometimes was not very clear. If, as it seems, non-caecotrophe faeces constituted an important proportion of the faeces used, this might be the first time that non caecotrophe faeces have been shown to play some role in communication.

The counter-marking of urine cues found in the experiments reported here provides strong evidence that faeces play a role in social communication by wild rats. The tests reported in section 3.5, 3.6 and 4.8, showed that rats discriminated between urine from residents and other colonies, and, although they counter-marked both of these with urine, they only faecal marked the latter. This specificity in the response, along with the finding that females only faecal marked in response to urine from other females, but not from males, strongly suggests that rats were responding to the type of urine. The pattern of urine marks and the quantity of urine deposited on the clean tiles serving as stimuli were unlikely to differ between colonies. This is the first instance where rats have been shown to respond to chemical cues by faecal marking. Bell (1980) found that rabbits show a similar response as dominant individuals defecate over conspecific urine samples. Hesterman and Mykytowycz (1968) found that humans classified the faeces deposited in response to conspecific odours as having a stronger odour than standard faeces, suggesting that additional olfactory information may be added to faeces when counter-marking. It is possible that there may be similar differences between counter-

marking faeces and other faeces in rats, particularly as rats also have anal glands (Schaffer, 1940; Montagna and Noback, 1947).

Another indication that rats may use faeces for communication is the finding that they formed latrines. Although latrines may be formed for hygienic reasons, as a means to keep faeces far from nesting areas (section 3.2.5), the observation that rats often sniffed at faeces, and the findings of the experiments reported in sections 3.4, 3.5 and 3.6 suggesting that faeces are used in social communication, suggest that latrines might be faecal marking posts. This would be consistent with the roles that latrines play in other mammal species (section 1.4.7.2.11). Environmental constraints on olfactory communication may have forced rats to increase the transmission efficiency of olfactory signals carried by faeces (Alberts, 1992) by creating latrines as visually conspicuous clusters of faeces or to increase the concentration of olfactory cues. The role that latrines may play is discussed in more detail in section 5.2.4.

The nature of the olfactory cues in their faeces remains to be unravelled. Possible candidates could be a bile salt produced by the rat, traces of hormones (as found in maned wolves, Wasser, De Lemos Velloso and Rodden, 1995) or some other chemical produced by the rat, perhaps altered or released by bacterial breakdown, or a mixture of some of these alternatives (as suggested by Brown, 1995). In the case of maternal pheromone, Leon found that neither bacteria nor the anal glands were involved in its production. Because the type of message carried by faeces in counter-marking seems to be an aggressive warning towards intruders, probably allowing the assessment of the competitive capability of the resident, the semiochemical/s involved is likely to correlate with this competitive capability. Thus it is unlikely that such semiochemicals

are dependent on the diet as this can change, and they are more likely to be chemicals dependent on testosterone or some biochemical indicator of size or aggressiveness.

5.1.1. The role of faecal marking in warning competitors.

The results from the experiments on faecal marking in response to urine marks from non-resident wild and laboratory rats (sections 3.5, 3.6) suggest that the primary role for faecal marking *in the context of these experiments* was to advertise the presence of the residents to competitors. However, it is important to note that this role does not exclude the possibility that, at the same time, faeces may attract mates, or be used in mate choice. The evidence suggesting a role for faeces in competitive advertisement is the following:

Rats faecal marked in response to urine marks from other-colonies (neighbours or unfamiliars), but not in response to their own urine cues or those belonging to their mate (resident urine cues were a mixture marks from both residents). This finding is not conclusive evidence for a role in communication between competitors in itself because the same results would be expected if faeces played a role in sexual communication (mate attraction, mate assessment, etc.).

Presumably, rat donors urine marked the tiles serving as stimuli as part of their territory. As explained in section 4.2 and 4.3, rats urine mark their home, producing a urine background similar to that found in mice (Hurst, 1987, 1989, 1990a, 1990b, 1990c). These urine marks might constitute territorial marks. Rats responded to clean areas ('gaps') in the odour profile by increased urine marking. Most of these urine marks belong to a male (section

4.4), the individual most engaged in the defence of the territory and in attacking intruder rats (section 1.4.5.1). Thus, placing an intruder's home range marks in the resident's home pen probably represented a challenge for the resident male and/or the female. By faecal marking this tile rats may be indicating to other rats that no challenge is ignored. However, it could be argued that despite using urine range marks from non-resident rats as a stimuli (which are likely to play a role in communication between competitors), residents may have responded to those intruder territorial marks as a sexual signal rather than as a mark of threat.

The faecal marking response was mainly directed towards stimuli from individuals of the marker's own sex. That is, males tended to faecal mark unfamiliar male urine more than unfamiliar female urine while females only marked in response to unfamiliar female urine (section 3.6). This is one of the responses expected if a scent is involved in communication between competitors (section 1.3.2.2.1.5). This type of response is particularly expected because, due to the reasons explained in section 2.6, sub-adult rats were used as donors.

Females were never observed to faecal mark in response to male urine. If faecal marking served to attract mates, we would expect males to faecal mark mainly in response to female cues, and females mainly in response to male marks. The lack of response by females towards males might be due to the fact that laboratory donors were immature or because female residents were not sexually receptive, as most female subjects used in the experiments were either pregnant or lactating most of the time. However, this interpretation does not explain why females faecal marked unfamiliar female cues nor why they investigated female more than

male urine (section 3.6). Finally, a sexual interpretation does not explain why the resident male was interested in male more than female urine (although only slightly more).

Males faecal marked urine cues from both sexes, but females did not. If faecal marking was a sexual display, it would be very difficult to explain why females were not interested in males and never marked in response to them, but they did in response to females. It seems more likely that females regarded other females as competitors. In addition, because urine donors were immature, wild resident females may have regarded immature females as unable to match their fighting abilities and responded to them with aggressive marking. In contrast, their lack of response to males may indicate a cautious response to males even with poor fighting abilities. Another possibility is that wild resident females were not receptive. Thus, they would avoid attracting males towards their scents and themselves and prevent a likely harassment. However, a similar lack of response should be expected towards females (considering a sexual response). In contrast, this latter response makes sense if females were warning their greatest competitors, which are usually females competing for breeding opportunities (section 1.3.2.2.1.4). The reason why males faecal marked females at a rate only slightly lower than that towards males may be due to competition alone or together with sexual communication. Males may have faecal marked in response to male urine as a threat warning, whereas their response to females may constitute a sexual display (which is not very likely because laboratory females were immature). However, it seems more likely that the resident male regarded unfamiliar females as competitors for resources or a danger to the male's offspring,

rather than potential mates, and faecal marked their urine as a warning.

Although a simple sex bias in faecal marking might be explained as a consequence of a greater body weight and a higher metabolic rate in males, that would not explain why females only faecal marked in response to female marks. Furthermore, the literature is unclear about which sex, if any, produces more faeces: female laboratory rats were found to ambulate and deposit more faeces than males (Gray and Lalljee, 1974), but laboratory males were found to produce more faeces when scared (Viveros, Hernández and Gallego, 1990).

Heavier males faecal marked much more than lighter males. In these experiments, faecal marking might constitute a high level warning prior to aggression, or a substitute for aggression when an intruder is detected but not found. A prediction derived from this hypothesis is that males that are more likely to win a contest should faecal mark more than males that are likely to lose. In the absence of any other indicators such as age, weight is the best predictor of fight outcome (Robitaille and Bovet, 1976). In the experiment on faecal marking towards urine cues from neighbours (section 3.5), there was one pen where faecal marking was greater than in neighbouring pens in both years when the experiment was conducted. Table 3.3 (section 3.5.4) shows that, in each case, this pen contained the heaviest male. Differences in absolute weight among males could not account for the results: the marking rate was 36.2 ± 3.3 faeces/trial for a male weighing 370 g (the heaviest in the first year). The following year a male weighing 347 g (only 53 g less) had a faecal deposition rate of 1.8 ± 0.4 faeces/trial. However, the largest male the second year, weighing 575 g, had a marking rate

similar to the heaviest male the year before (30.4 ± 3.2 faeces/trial). These results suggest that exchanging tiles between enclosures probably produced a chemical contest. The mechanism might have been the following: the rats might have used chemical cues carried by urine to assess their chances of winning a contest with their neighbours; subsequently, those males with the highest fighting ability would be more likely to display a high level threat (i.e. faecal counter-marking) towards those males whose territorial marks he found and which he would be likely to defeat easily. In order to corroborate this hypothesis, a study of the relative dominance between pairs of male rats was needed but, unfortunately, it was not possible to carry this out.

As yet, there is no evidence that rats can discriminate competitive ability from faeces. However, there is some evidence that rats with different competitive ability differ in their rate of *urine* marking (Taylor, Bartko and Farr, 1987), and that rats can discriminate soiled bedding (probably containing urine in addition to other scents) from dominant and subordinate rats which differ in their competitive abilities (Krames, Carr and Bergman, 1969).

5.1.2. Other possible roles for faecal marking.

The role an olfactory signal plays may depend on the context and the way in which it was deposited. Urine and faecal marking may be used in other contexts as sexual attractants (or if the urine donors are receptive females), but the challenge of intruder home range marks found in a resident's home range in the present experiments may override any role for faecal marking other than warning competitors. In addition, a strong display and intolerance of intruders or challengers may serve, at the same time, to attract

mates that are in a suitable receptive condition, and assert the dominant status of the marking individual in front of other members of the colony, thus serving as an internal group display in addition to being a display to intruders.

Further experiments are needed to assess whether faeces play a role in sexual communication. One might be the advertisement of female receptivity. These experiments will need to test whether male rats investigate faeces from oestrus females more than those deposited in other stages of the cycle, or whether urine marks deposited by an oestrus female trigger more faecal marking by males than urine marks from a non-oestrus female. It would also be interesting to compare the male response to adult and juvenile females. The possibility that faeces play additional roles in sexual communication (section 1.3.2.2.2) should also be considered.

It is, however, not obvious why should rats use faeces in the advertisement of sexual receptivity. Pro-oestrus and oestrus last about one day in rats. Urine and sebum seem to be more suitable as media for a signal designed to last for such a short period of time, as indeed, has been found in the literature (Calhoun, 1962; Birke, 1978; Birke and Sadler, 1984; Lee Mitchell and Adams, 1984; Matochik, White and Barfield, 1992; but see Peden and Timberlake, 1990). Faeces, however, provide a longer lasting signal (Alberts, 1992), and appear to be most suited to home range marking (in badgers: Roper, Shepherdson and Davies, 1986; Roper et al., 1993; in rhinoceroses: Owen-Smith, 1971). However, faeces may play other roles in sexual communication such as assessment of male quality (using the same olfactory cues that allow intruders to assess the resident male's fighting ability).

Because rats produce more faeces when scared or stressed (Harkins, Becker and Wright, 1974; Gentsch, Lischtsteiner and Feer, 1981; Gentsch et al., 1982; Viveros, Hernández and Gallego, 1990), it is possible that faeces could carry a message of stress or fear to other rats. Although urine left by stressed conspecifics alone or with faeces slows down naive rats running in an alley maze, faeces alone fail to produce such effect (MacKay-Sim and Laing, 1981a). This suggests that faeces do not convey stress messages but perhaps they might in different circumstances. In MacKay-Sim and Laing's (1981a) study, the rats deposited faeces after they had been stressed but which had been formed in the intestines prior to the stress situation. However, longer term stress might alter the composition of faeces *while they are being formed* in the gut and thus these faeces, voided later, might carry a stress message. Such changes may be mediated by hormones. Sexual hormones have been found to induce cyclical changes in populations of bacteria living on the rat genitalia (Larsen, Markovetz and Galask, 1977). Hormonal changes produced by stress may alter the bacteria flora in rat intestines and subsequently, the scents produced by faeces.

It has also been suggested that faeces convey information about the food eaten by a rat (Laland and Plotkin, 1991). Although they appeared to induce other rats to select the diet eaten by the rats depositing the faeces if these are found together with other scent marks, Laland and Plotkin (1991) found that faeces failed to induce diet preferences on their own. If faeces induce diet selection, they might be expected to be conspicuously deposited in feeding areas. Although that was the case in Laland and Plotkin's report on albino rats kept in small cages under laboratory conditions, such clustering of faeces was not found around feeding bowls in the enclosures. On

the contrary, feeding bowls were usually either devoid of faeces or they had one or two faecal boli around them. This apparent contradiction may be a consequence of the possibility that faeces might attract the attention of predators. Wild rats might avoid the risk of predation by not depositing faeces at feeding points. Rats spend long periods at such points and depositing faeces may induce predators to target these sites. Alternatively, faeces might carry information about the diet eaten by the marking rat even though they are not deposited near feeding points, although in such a case their efficiency in the social transmission of diet preferences might be reduced. Perhaps rats can learn about diets during the brief periods of faecal investigation at latrines, where they are more protected than in open feeding areas. An alternative explanation may be that coprophagy was more common in wild rats, or in more naturalistic settings. In this case faeces would influence diet selection at feeding sites as Laland and Plotkin (1991) suggested, but they might be quickly eaten resulting in the observed lack of faeces. However, I was unable to detect such a response during the period of observation reported in section 3.2.1.

5.1.3. Why use faeces to counter-mark.

An important question arising from the experiments is why the rats used faeces to counter-mark instead of simply using urine.

The introduction of urine marked tiles triggered both faecal marking and an increase in urine marking (sections 3.5 and 4.8). It seems unnecessary to use two types of scents to counter-mark in the same context, which suggests that they both have the same purpose. There are, however, several possible explanations, which are not mutually exclusive:

-Faeces release volatiles more slowly than urine, because they have a reduced surface area compared to their volume. Additionally, faeces may contain a greater amount of scent or substratum on which bacteria could act continually to produce volatiles. Therefore, by using faeces, rats may ensure a longer lasting message. Since the amount of urine marks deposited by non-residents on the tiles (and hence probably the challenge) was great, a longer lasting signal such as faeces may be more effective in signalling territorial onwership (Alberts, 1992) than urine alone.

-Faeces are more conspicuous than urine and thus they are more likely to attract the attention of an intruder than urine alone (Alberts, 1992). In fact, in many trials, groups of 20 to 30 faeces deposited as counter-marks were common and highly conspicuous.

-Faeces may constitute a stronger warning than urine. Urine might have been used to mask the opponent's marks (as happens in golden hamsters: Johnston, Chiang and Tung, 1994; Johnston, Munver and Tung, 1995) whilst faeces might indicate a stronger threat of aggression. Different scents from the same individual have been found to have different roles (Ferkin and Johnston, 1995a). Urine and faeces may carry different information, and thus they may have slightly different functions. Although both seem to be used for the same purpose (sections 3.5.5, 3.6.5 and 4.8.4), faeces may carry more information than urine about the size, health or social rank of the donor. Urine, for example, might be used for general home range marking and counter-marking occasional marks found. Faeces, in contrast, might be explicitly used for high-level challenges, similar to the way that matching the opponent's song type, strophe length and delay in bird's songs constitute a greater, more specific threat than a simple song (McGregor et al., 1992).

Gosling et al. (1996) have argued that, when the fighting ability of an intruder matches or is greater than that of the territory owner, the intruder should seek more specific information than that given by territory scent marks. Faeces may provide such a source of more detailed information about a territory owner. Thus, rats may faecal mark in order to provide additional information to an intruder that has indicated that it has not been deterred by depositing its own marks in the residents' territory.

5.1.4. Rats form latrines.

Rats form latrines which, as discussed in section 3.4, are not a product of incidental deposition. Why should rats form latrines? The possibility that latrines may play a role in social communication or are created for hygienic reasons has already been pointed out (section 3.3.4). This section will discuss, in greater detail, the social roles that latrines may play and also will examine the possible reasons why no rat latrines have been mentioned in the literature.

Latrines may serve as marking posts where only the dominant male marks. Latrines would then be sites where all individuals, intruders included, could assess the identity and perhaps other characteristics of the dominant male. This may serve a number of functions: mates might be attracted while competitors might assess the characteristics of the territory owner and, in most cases, would probably be deterred. Latrines may also be 'bulletin board sites' for the exchange and update of information between all the members of a colony. If so, all colony members would be expected to contribute to the build up of latrines in a similar manner. It is also likely that latrines may serve for both purposes to some extent. For example, they may be formed mainly by the dominant male to

warn off intruders and/or to seek mates, but other individuals may still contribute in small proportions to gain tolerance by the dominant or exchange information.

To test these hypotheses in rats, I designed an experiment which assessed sex bias in faecal deposition at latrines (see appendix A) compared with deposition elsewhere. Unfortunately, during isolation, wild rats were extremely reluctant to eat from the cage feeder which contained dyed food, and the faeces were not dyed. To feed each wild individual rat separately with a different dye without causing disruptions in the social life of the colony appears to be extremely difficult due to the isolation needed and the difficulty of inducing them to eat the dyed food in such stressful circumstances. Alternatively, DNA analyses might be carried out on the faeces at latrines and elsewhere to identify donors and examine the faecal contribution of each individual towards latrines. This type of experiment should be of primary importance to unravel the role that latrines play for wild rats.

Reports of faecal marking and the building up of latrines in the literature seem to be related to territorial marking in most cases.

Rabbits produce latrines both under experimental and natural conditions (Bell, 1980, 1981; Veberne and Blom, 1981; Sneddon, 1991). Males produce latrines in captive conditions, and some males defend these areas (Veberne and Blom, 1981). Similarly, the presence of faeces and anal gland secretion have been found to increase the probability of a rabbit winning a fight (Mykytowycz, 1973; Mykytowycz et al., 1976), which might happen because the rabbit recognises the area as its territory and thus is more prepared to defend it than if it is an unfamiliar area. Male rabbits possess larger anal glands than females and it is primarily the males (and

most of them) which make use of the secretion of their anal glands in marking territories and maintaining latrines, although females also faecal and urine mark (Mykytowycz and Gambale, 1969; Mykytowycz, 1970).

In rhinoceroses, faecal marking also seems to be related to defence of the territory (Owen-Smith, 1971). Territorial bulls faecal mark at latrines. After defeat, a previously dominant bull ceases to both urine and faecal mark.

In hippopotami, latrines are also produced by all males and not by females (Klingel, 1991). Dominant individuals display ritualised faecal marking in front of each other during boundary disputes. Perhaps the faecal marking found in my experiments in response to unfamiliar urine marks may reflect chemical disputes similar to those accompanying physical contact in hippopotami.

Badgers also seem to use latrines as a form of territorial marking. There are two types: those placed around the borders of the territory and are formed mainly by males, and hinterland latrines used by both sexes (Roper et al., 1993). Roper et al. (1993) found that this species also produces clusters of faeces inside their territory (termed temporary defecation sites instead of latrines). These are produced by both sexes but Roper et al. (1993) hypothesise that these play no role in communication.

In some species latrines seem to function as information exchange centres or sites to acquire a group odour. This appears to be the case in rabbits (in addition to the territorial role discussed above). Sneddon (1991) found that female rabbits visit latrines and sit for a few minutes before leaving, probably to acquire a group odour.

There appears to be no mention in the literature about a role for latrines in mate attraction, although perhaps the reason is that little attention has been drawn upon this role. Only in the case of ferrets, *Mustela furo* (Clapperton, 1989) do we know that female faecal marking does not increase at oestrus. If faeces at latrines carry information about the physical condition, resource holding potential or fighting abilities of the dominant male, they may also serve to attract mates and serve in mate choice as much as to deter competitors. This possibility does not seem to have been tested in any species.

If latrines constitute territorial marking posts, faeces deposited in latrines may be different from those deposited elsewhere. This is the case in rabbits (Bell, 1980). The difference between faeces at latrines and other places seems to be due to a higher amount of anal gland secretion deposited on faeces at latrines (Sneddon, 1991). This could be a mechanism used by rabbits to convey information about the higher fighting abilities of the dominant male. In the case of the rats, no obvious difference between faeces at latrines and elsewhere was apparent to the human observer. However, further experiments may prove that rats can discriminate between them.

Rats formed latrines in similar sites to other species. Rabbits, for example, produce latrines in corners when kept in large enclosures (Veberne and Blom, 1981). Ferrets build latrines close to the nest when captive (Clapperton, 1989), although, in contrast, they tend to faecal mark the centre of the enclosure more than the corners. As among rats, hippopotami produce latrines under shelter, in this case under bushes instead of at corners (Klingel, 1991). Also similar to rats, hippopotami do not form latrines in feeding areas, although they build them on pathways.

No mention of rat latrines, apart from Calhoun's groups of faeces, was found in the literature (Section 1.4.7.2.11). Why is this when they are so visually obvious (section 3.2) and there is such a large number of papers on rat behaviour? Much of the behavioural research on *Rattus norvegicus* has been conducted in the laboratory. Under such conditions, hygiene is essential to keep infections to a minimum and prevent the build up of high levels of ammonia (Poole, 1987). Therefore, cages are cleaned every few days. This means that faeces are swept away before they may form latrines. In the experiments reported here, enclosures were cleaned only after each colony was removed, as doing it more often would have resulted in a great disturbance for the rats.

There is another argument for not expecting latrines to be formed in cages. Faecal marking has been related to competitive advertisement and aggression towards intruders. However, aggression increases when the degree of captivity (that is, the amount of space per rat) is increased (Boice and Adams, 1983) while dominance relationships are established in large indoor/outdoor pens, they are not in laboratory cages (Adams, 1985). If latrines are produced only under conditions where dominance hierarchies are firmly established and intruders would be chased out of the territory, then latrines would not be expected to appear in small laboratory cages. Additionally, rats may need a large home range to form latrines. A laboratory cage (usually about 0.25 m^2; see section 2.1) may be too small to require latrines for chemical communication. That would be the case if, for example, latrines served as a site for exchanging chemical information about individuals who may not meet very often.

A further reason which may explain the lack of reports of rat latrines is that most research has been carried out using laboratory strains. Wild rats seem to be more aggressive than laboratory strains (section 1.4.3). Perhaps latrines and faecal marking are only formed by highly aggressive individuals. This might also explain why no faecal marking has been reported in either indoor or outdoor colonies of laboratory rats (Boice and Adams, 1983; Adams 1985) even in studies where urine marking or other type of scent marking was reported (Boice, 1977).

Faeces are difficult to detect in the wild (personal observation). Reports on wild free-ranging rats (Telle, 1966; Robitaille and Bovet, 1976) may have missed latrines since the colour of faeces is not likely to be conspicuous against dark soil without vegetation. Rats may form latrines in covered areas without ground cover vegetation as the only latrine detected in the wild was found under a tree where no such vegetation grew.

A final alternative is that other researchers did not consider scent marks in their studies, misinterpreted latrines as artefacts of captive conditions, or they simply failed to understand their potential significance.

5.1.5. Faeces as honest signals.

Faeces may be honest signals, i.e. impossible to cheat. Faecal marking seems to play a role in warning intruders, possibly about the aggressive intentions of the marking individual. As discussed earlier, some males deposited around 30 faeces/trial in response to marks from rats belonging to other colonies (see table 3.3, section 3.5.4). Such a large number of faeces may constitute an honest signal regarding the fighting ability of the resident because:

i) A rat has to eat and metabolise a large amount of food to produce a large amount of faecal material. Consequently, the rat has to have access to abundant food. Dominants have preferential access to food (Smith, Smith and Sibly, 1991; Thullier et al., 1992), which may reduce the intake rate of subordinates and floaters (wandering individuals which do not belong to a group of rats or hold a territory).

ii) If the amount of faecal material produced is correlated to body size, only the largest rats will have the body size required for a meaningful faecal marking response (which may be constituted by 30 faeces per night as mentioned above). These are likely to be the dominant rats (Robitaille and Bovet, 1976), although, as mentioned before, body size does not always correlate with dominance (Berdoy, Smith and Macdonald, 1995; Berdoy, Webster and Macdonald, 1995). Small cheaters are unlikely to mimic the faecal marking rate of an authentic dominant rat due to their size.

iii) The ability to produce a large quantity of faecal material may also indicate a high metabolic rate. Thus, the marking individual might be able to afford greater expenditure of energy in fights than one which is not able to achieve such a high rate of production of faecal material, and therefore it will be more likely to win a contest.

iv) Perhaps faeces are a high level threat. If so, cheaters are likely to incur such a high cost if eventually forced to fight that it would not compensate them to cheat even if they were physiologically able to do it.

These explanations are not mutually exclusive and more than one mechanism may be in action at the same time.

Faecal marking may have evolved in rats because it may be a signal more difficult to fake than urine marks. Urine might be used to indicate the presence of residents in a territory whereas faeces might be an unfakeable means of assessing the fighting ability of contestants. Alternatively, urine might be a general purpose signal that gives a small amount of information about the size and fighting ability of the residents, whereas faeces give much more detailed information. In which case, if urine is energetically much cheaper to produce than faeces, urine would be used for general scent marking and warning of intruders, whereas faeces would only be used when the residents are challenged. Another reason why faeces may be an honest signal, or at least, one better than urine, is that, unlike urine, faeces are very easy to remove. Thus, if a set of faeces is present in a territory, they are likely to belong to the dominant individual, as they would have been eliminated if the faeces belonged to any other individual. Alternatively, faeces may have evolved as a method for scent marking because they are a method of scent marking longer lasting than urine.

5.2. Urine marking.

5.2.1. The pattern of urine marks.

Rats eventually covered all the tiles placed in their enclosure with urine marks (4.2.3). The test introducing clean tiles and tiles urine marked by non-resident rats showed that urine marks are not the product of incidental marking, but instead rats appear to create a background of urine marks that allows them to detect at least major changes in the odour profile (such as a clean area and a site abundantly urine marked by rats from other colonies). This seems to be similar to the system used by house mice (Hurst, 1990a, 1990b,

1990c). The large increase in urine marking in response to clean tiles may be an effort by the rats to keep the urine background stable. Urine marks might assist rats in orientation, as the experiment with clean tiles suggested that the background of urine marks might help the rats to detect unfamiliar objects (devoid of residents urine marks). The background of urine marks may play an additional role in orientation: it may also signal home for the rats. By monitoring the odour profile rats can probably detect the limits of their home range. Similarly, intruders might detect that a territory is occupied. Also if, as some authors claim (section 1.4.7.2.9), rats can detect odours from stressed rats, urine marks deposited by frightened or stressed individuals may also signal which areas to avoid.

The distribution of urine marks found in the enclosures was not uniform, as described in earlier studies (Calhoun, 1962; Telle, 1966; Barnett, 1975; Boice, 1977). Wild rats in the enclosures, as in studies with laboratory rats (Peden and Timberlake, 1990), usually moved along the walls, and, less frequently, around the centre. Also in agreement with previous reports (Richards and Stevens, 1974; Peden and Timberlake, 1990), the density of urine marks was higher along the walls than elsewhere. Calhoun (1962) reported that rats feel attracted to vertical surfaces, whereas Taylor (1978) observed that they usually move within or close to cover. As discussed previously (section 4.2.3.4), the greater urine marking by the walls may correspond to the urine trails described by Calhoun (1962), Telle (1966), Boice (1977), and more recently, the foraging trails found by Galef and Buckley (1996). Although Telle (1966) reported that these trails attracted both resident and naive rats, there is no evidence that the semiochemical producing this

attraction is in urine (section 5.1.2). One of the mechanisms by which urine trails may assist rats in orientation may be as follows: if, as mentioned before, urine marks assist rats in orientation by indicating the degree of familiarity of an object or area, then the higher density of urine marks in the trails and other areas around the nest may indicate to the rats that such areas have been recently and frequently visited by others, and therefore, that the path is safe and obstacle-free.

5.2.2. Comparison between the distribution patterns of urine and faeces.

The different distribution pattern of faeces and urine suggests on the one hand that they are not a by product of the activity of the rats, and, on the other, that urine and faeces do not play exactly the same roles. The finding that faeces are concentrated in a few restricted areas under some sort of cover whereas urine marks are deposited in all areas of the enclosure may suggest that the information being extracted from faeces is more detailed (or difficult to acquire) than that from urine marks, requiring a longer investigation time. That would explain the need to build latrines in shelter areas where longer investigation times would incur less risk of predation than in open areas. In addition, the fact that urine forms a tapestry of marks suggests, as previously discussed (previous section), that urine might assist in orientation. It is less likely that faeces play a similar role considering their more restricted distribution.

5.2.3. The role of urine marking in social communication.

My results confirm findings from other studies indicating that urine marks play a role in social communication, and suggest that one role may be to warn intruders. The evidence is based on marking responses to conspecific urine and also the sex differences in urine marking.

It has already been discussed that females mark more at pro-oestrus and oestrus (section 1.4.7.2.6), which suggests that urine plays a role in advertising the receptivity of females.

The results of the experiments reported in section 4.4 showed that wild male rats have a greater rate of urine marking on clean tiles than females. Although differences in marking between males and females might be explained in terms of metabolic differences, it should be noted that these metabolic differences might be needed to secure the production rate of faeces and urine needed for communication (see section 1.4.7.2.3.). Furthermore, potential physiological differences between males and females cannot explain why rats would investigate urine from their own sex for longer than that from the opposite sex or vice versa (section 3.6).

A greater rate of urine marking by males might be explained by both a sexual communication hypothesis and a competitive communication hypothesis.

-Males may be expected to mark more than females if they are advertising for mates. Females are either pregnant or lactating most of the time (Calhoun, 1962; author, personal observation), and would not be expected to advertise to potential mates during these periods. Urine marking might also be a sexual display by the sex which invests less in offspring. In this case, chemical cues may

reflect the mate quality of the male. One set of compounds involved in this might be the proteins excreted in the urine, α_{2u}-Globulin in rats (Vandoren et al., 1983) or MUP (major urine proteins) in mice (Robertson, Beynon and Evershed, 1993). Male laboratory rats produce considerably more proteins in their urine than females, even when weight differences are taken into consideration (Finlayson and Baumann, 1957; Vandoren et al., 1983). In addition, α_{2u}-Globulin in males is testosterone dependent (Vandoren et al., 1983), as are aggression and fighting abilities (section 1.4.7.2.8), which are likely to correlate with the resource holding potential of a male.

However, if sexual advertisement and mate attraction were the only roles urine marking plays in social communication, then males would be expected to investigate urine from females more than that from males. The results of the experiment in section 3.6 showed, in contrast, that males investigated urine marks from male laboratory rats more than those deposited by females. This suggests a competitive role for the urine marking response and investigation in my experiments, which is not surprising in view of the fact that the laboratory rat donors were immature (for reasons explained in section 2.6), and therefore unreceptive as mates. Moreover, considering the immaturity of the donors, if the only role that urine plays is a sexual one wild female rats should not have discriminated when investigating marks belonging to males and females. In contrast, the results show that females investigated female cues for longer. In addition, the immature laboratory females readily urine marked the tiles in their cage. This suggests that urine marks, at least in the context of the experiment cited, play a role in communication between competitors.

-Males may mark more than females to advertise their dominant status. It has already been discussed in section 1.4.7.2.5 that urine marking and aggression are correlated. The dominant male may mark the home range more than females do in order to warn intruders that the area is occupied, or to provide intruders and residents with a mechanism to associate the individual territory owner with the defended area (Gosling, 1982), in which case the lower rate of female urine marking might serve to induce dominant male tolerance (Hurst, Barnard and Fang, 1993), as males may attack unfamiliar or neighbour females (section 1.4.5.1). This hypothesis is consistent with the differences found in the time rats spent investigating scents from unfamiliar male and female rats. Males may investigate male more than female urine marks because individuals of the same sex tend to compete more than individuals of opposite sexes. As a result of this, the information individuals gather from conspecifics of their own sex may be more detailed than that from the other sex. For example, males might be interested in the social status and body condition and characteristics (size, age, etc.) of another male in order to assess the most likely outcome of a confrontation. In contrast, males might only be interested in gender assessment and reproductive status of females to know if these are willing to mate. As discussed in section 5.2.1, because the tiles serving as stimuli in the experiments reported in sections 3.5, 3.6 and 4.8 were marked as part of their home range by rats from other colonies, this is likely to have produced a challenge for the resident rats. This challenge may have been even greater when immature laboratory rats deposited the marks because their fighting abilities were, presumably, far poorer than those of the resident wild rats. Therefore urine donors were more likely to be considered as

intruders than as potential mates (particularly in experiments where donors were immature laboratory rats). Clean tiles would then have been marked to advertise occupancy, because any unmarked area may be prone to be occupied by alien rats. In addition, dominant males may be advertising their social status through an increased urine marking rate (Taylor, Bartko and Farr (1987) found the more aggressive a male is, the more it urine marks). To test this hypothesis, it would be very interesting in future experiments to compare the marking rate of subordinates and dominant males and a dominant male's response to a large concentration of subordinate's marks.

In the choice test presenting fresh marks consisting of urine from either residents or neighbours, rats deposited about twice as many marks on the latter than on their own marks. Since, at least in hamsters, it has been found that depositing scent marks which either totally or partially cover previous marks can result in the masking of previously deposited scents (Johnston, Chiang and Tung, 1994; Johnston, Munver and Tung, 1995), rats might be trying to make urine marks from non-resident rats unavailable for investigation to any rat (be it resident or from other colony). The reason may be that, by masking their rival's signal, the dominant shows that he is not going to use that information nor is he going to allow others to use it (McGregor et al., 1992). The results from this experiment (section 4.8) also suggest that urine marking plays a role in communication between competitors. However, the possibility that male urine marking was stimulated by the minute amount of female marks present on the neighbour-marked tile cannot be ruled out. Again, such a sexual response would not explain the greater time that rats spent investigating marks deposited by individuals of

their own sex (section 3.6). An experiment monitoring the sex bias in urine marking (as in section 4.4) in response to marks from either male or female unfamiliar rats might add essential evidence supporting a role for urine marking in communication between competitors.

However, it is important to remember that the roles in orientation and social communication discussed here are not mutually exclusive. Therefore, rats may be using urine marks for several purposes simultaneously.

5.2.4. Urine as an honest signal.

If a high urine marking rate could deter competitors and establish the dominant status of an individual, why do not all members of the colony try to cheat? One reason, as indicated by Inglis and Shepherd (1990), may be that the cheater would not be able to bear the costs of its deceit: if it is a subordinate, marking at a rate similar to that of a dominant individual may trigger the dominant's aggression, as is the case in mice (Hurst, 1993). In contests with intruders, the cheater would incur a greater risk of injury in fights because any individuals deciding to escalate a fight would have fighting abilities similar to those deceitfully displayed by the cheater, but much greater than the real fighting ability of the cheater. Additionally, the semiochemicals used to signal social status or to warn intruders might be impossible to fake. For example, the α_{2u}-Globulin in rat urine and the major urine proteins in mice (MUPs) apparently involved in chemical communication appear to be testosterone dependent (Vandoren et al., 1983; Robertson, Beynon and Evershed, 1993). Because in some species such as rats, testosterone titres are higher in dominant rats

(Blanchard, D. C. et al, 1993), these proteins may only be present in a sufficient quantity to be effective in dominant males. Also, Drickamer (1995) found that the urine production rate of dominant male mice is greater than that of subordinates. Subordinates may not be able to produce enough urine to advertise a status higher than that they hold (e.g., as a consequence of a smaller body size, decreased physiological capability, etc.). Alternatively, the energetic costs of urine marking the home territory frequently may be too large for cheaters (e.g. if they did not have access to as much food as the truly dominant individual).

5.3. Marks deposited by the rats and marks deposited by the researcher.

The results from the choice test on faecal discrimination (section 3.4), do not seem to fit any of the functional hypothesis considered comprehensively (section 3.4.4). Similarly, rats did not seem to respond to a urine streak on a clean tile (section 4.7). In both experiments, urine and faeces were collected and deposited by the experimenter. Other experiments involving urine collected and placed by the experimenter, and differing from the latter in procedure and stimuli, also obtained weaker or no response (sections 4.5, and 4.6). In contrast, responses in experiments where the rats deposited the urine marks themselves were remarkably strong. This suggests that rats may respond differently to artificial stimuli than to cues deposited by the rats themselves (here referred as natural marks), although the implications of using naturally voided scents or those applied by the researcher do not appear to have been considered before in rat studies. Only one published report (Birke and Sadler, 1984) found a different response of laboratory rats

towards marks placed by the researcher (artificial marks) and deposited by rats (natural marks), although the authors did not seem to be aware of the possible reason for this.

There are two possible alternatives to explain these results: the difference might be either in the composition of the urine or faeces deposited, or in the distribution pattern. I will discuss these alternatives regarding the experiments conducted here.

The composition of the scent may be different in artificial marks compared to natural marks for two reasons: i) stress during collection of the scents used or ii) rats change the composition of scents according to the behavioural context.

-Stress during collection. Urine and faeces were collected whilst the rats were kept in small individual cages. Wild rats were scared and stressed while in cages: they often squeaked and chattered their teeth, and jumped towards the experimenter crashing against the cage. Perhaps during the choice tests rats responded to cues regarding stress, which overrode the meaning the marks would have had had they been deposited by the rats under more natural circumstances (section 3.4.4). Stress can produce a number of physiological changes including alteration of hormone titres (Blanchard and Blanchard, 1990; Blanchard, D. C. et al., 1993). These can affect the bacterial flora (Larsen, Markovetz and Galask, 1977) which, in turn, could affect scents through chemical breakdown (Albone, 1984). For example, hormones can be detected in the faeces of maned wolves directly through biochemical assays (Wasser, De Lemos Velloso and Rodden, 1995). If hormones similarly are present in rats' faeces and are detectable by other individuals through olfaction, they may be able to detect hormones such as adrenalin or corticosterone which are produced in increased

quantities under conditions of stress. Finally, the production of proteins in the urine of both rats (Vandoren et al., 1983) and mice (Robertson, Beynon and Evershed, 1993) is dependent on sex hormones. In the case of mice the proteins known as MUPs, are also known to be involved in olfactory communication (Robertson, Beynon and Evershed, 1993).

-Artificial marks may also be different to natural ones because rats actively altered the composition of scents according to the social context (and not just passively as a consequence of stress). Thus, pooled urine or faeces collected from caged rats might not have a social message, in contrast to urine on tiles marked by laboratory rats as a part of their home range (section 3.6). Rabbits, for example, deposit faeces in response to conspecific odours which are different to other faeces (Bell, 1980). Faeces in latrines seem to have more anal gland secretion than those deposited by rabbits elsewhere (Sneddon, 1991). Similarly, meadow voles deposit scents (including urine and faeces) that evoke a sexual response only during the breeding season, and not during the rest of the year (Ferkin, Sorokin and Johnston, 1995). Rats might also change urine protein (MUP in mice or α2u-Globulin in rats) concentration or biochemical properties according to the social context in which the urine is deposited.

-The distribution and concentration of marks may also carry a message. In many reports where a small spot of urine was used as stimulus, rats investigated male urine longer than clean substratum (Brown, 1975, 1985c, 1986c, 1991; Birke and Sadler, 1984). However, Gawienowski, DeNicola and Stacewicz-Sapuntzakis (1976) found that rats preferred to stay in the clean half of a cage rather than in the half that had been sprinkled with adult male urine.

That is, artificial marks evoked opposite responses in different experiments. The pattern of distribution of the marks in the latter experiment may be one of the reasons for the avoidance of the marked area, although it might also be due to a greater amount of urine deposited in the latter case. Perhaps in my experiments, the way in which faecal and urine stimuli were deposited by the experimenter influenced the message they carried and thus, the response of the rats (although care was taken to place faeces in a pattern similar to that found in the latrines).

An additional possibility is that the collected urine or faeces were not the substance used in communication, but only the substratum for it. In this case, the response to natural marks would be greater because the biologically active chemicals are only found in natural marks. Chemical breakdown of scents by bacteria can alter or create their behavioural attractiveness (Albone, 1984). The urine triggering a response in the experiments from sections 3.5, 3.6 and 4.8 was deposited by the rats the previous night. In this case, the scent attracting the rats might not be from the fresh urine at all, but the mixture of aged scents impregnating the feet of the donor rats left when walking first on the substratum and subsequently on the tiles. A similar process might occur in communication mediated by faeces. Alternatively, the active semiochemical might be produced by foot glands, sebum glands or other scents.

5.4. Suggestions for future research.

Most of the chemical communication system in *Rattus norvegicus* remains to be discovered. Understanding this can help to elucidate how animal communication works. Comparing the olfactory communication system used by rats with those of other

mammal species and relating the differences found to the ecology of the species could provice considerable insight into the behaviour, ecology and evolution of the ubiquitous brown rat.

Faecal marking in competitive advertisement. Studying faecal marking in a colony including subordinates for comparison with the results reported here for pairs of rats would help to assess the role faecal marking plays in the rat communication system. One of the aims should be to assess the response of subordinates to faecal marking by the dominant male and female, to assess whether subordinates themselves faecal mark, and if they do, in response to which types of stimuli. This will help to elucidate whether faecal marking constitutes a status badge (e.g. individuals may faecal mark only in response to urine from conspecifics of similar or lower social rank) or whether it is related to communal defence of the territory (if faecal marking is stimulated only by intruders' marks). It would also be interesting to test whether the dominant male and female faecal mark in response to a high urine marking rate by subordinates (which might mimic a high status display), and also whether such a response would be accompanied by aggression towards the marking individual (whose known fighting ability would not match the displayed one). Such specific aggression towards a subordinate urine marking at high rate has been found by Hurst (1993) in mice. Because faecal marking might then be triggered by a familiar olfactory cue from one of the residents, these experiments would show whether faecal marking is used for intra-group communication in addition to being used to warn intruders (as shown in this thesis). Finally, it would be interesting to know how wild rats respond towards faecal marks from non-residents (i.e. faeces deposited in response to urine cues): whether they try to mask

the faeces with urine, whether they remove, chew them, etc., and whether such faecal marks elicit a response stronger than urine from non-residents. Faeces may be counter-marked with urine or just with fresher faeces, as seems to happen in badgers (Roper et al, 1993).

Comparisons could also be made to assess differences between faeces deposited in response to urine marking, those deposited at latrines, and faeces deposited in non-latrine sites. These comparisons might be biochemical analyses and choice tests presented to rats. The results may elucidate a rat's possible manipulation of the chemical composition of faeces (perhaps the amount of anal gland secretion), and thus, suggest the role that latrines and faeces play. For example, a greater amount of anal gland secretion and greater interest towards faeces from latrines or faecal marks compared with faeces from the same individuals scattered in open areas may suggest that the former are used in communication whereas the latter are not. Assessing the contribution of each individual to latrines, faecal marking, etc., and relating these differences to rat body weight, anal gland size, etc., may show which individuals are able to produce faeces effective in warning intruders, and therefore, to assess whether faeces are an honest signal (e.g., faeces produced for faecal marking may need a large amount of anal gland secretion, a large anal gland secretion and perhaps the only individual having this is the dominant male). Assessing a sex bias in the production of faeces may suggest a role for faeces in competitive or sexual communication (e.g. perhaps males produce many more faeces than females, perhaps both produce similar quantities but mainly males defecate at latrines, or there might be sex differences in volatile composition).

Faecal marking in sexual communication. Assessment of the role faecal marking may play in sexual communication requires greater knowledge. **Mate attraction:** manipulating the sexual status of female urine donors (oestrous versus non-oestrus) in tests of male faecal marking, and comparing the response of wild females in both stages to the faeces deposited by a male, might suggest whether faecal marking is used to attract mates. Assessing the response of males to female faeces may also help to elucidate whether the failure of females to faecal mark male stimuli is a response to avoid attracting unwelcome sexual attraction, or the result of fear of aggression from the intruder male. **Mate assessment:** Tests comparing the response of receptive and unreceptive females to faeces from dominant and subdominant males may suggest a role for faecal marking in mate assessment.

Faecal marking in other contexts. Faecal marking may also play a role in diet selection (Laland and Plotkin, 1991). Because rats produce faeces when scared or stressed (Harkins, Becker and Wright, 1974; Gentsch, Lischtsteiner and Feer, 1981; Gentsch et al., 1982; Viveros, Hernández and Gallego, 1990), they may also convey information about the emotional status of the donor. Experiments in this area should also be very interesting and fruitful. Moreover, results on the role of urine and faeces in stress may result in the design of new methods of rodent control, as these or semiochemicals carried by them may be used to scare rats away from buildings.

Latrines. The function that latrines play in the olfactory communication system of wild rats still remains to be discovered. The experiment shown in appendix A (assessment of differences between the male and female contribution to latrines) may be modified to assess the proportion of faeces from different individuals

in both latrines and non-latrine sites. DNA analyses could be carried out on faeces from latrines and other sites to identify their donors. The results of this experiment might show whether latrines constitute the marking post of the dominant male, and the contribution of other members of the colony to latrines. Monitoring the frequency and timing visits to latrines could provide insight into the possibility that latrines are involved in producing a group odour (e.g. if most individuals visit and stay in latrines often without defecating or investigating) or serve as bulletin boards for individuals to gather olfactory information (e.g. if individuals visiting latrines spend most of their time defecating or investigating them).

It would be of particular value to study latrines in the wild, despite the difficulties for conducting experiments under such circumstances, to see how well their constitution corresponds to those formed in enclosures. Thus, results obtained in the laboratory could be validated with field experiments.

Urine marking in competitive advertisement. More experiments are needed to assess the role that urine marking plays in the rat communication system. As discussed before, it would be very interesting to assess male and female urine marking in response to urine marks deposited by either male or female conspecifics. Close range monitoring like that used to assess the bias in urine marking (section 4.4) would probably be the best design for such tests. This would add evidence to support a role for urine marking in communication between competitors (as suggested by the investigatory differences reported in section 3.6) or in sexual behaviour. Assessing urine marking and investigatory responses of wild rats kept in large colonies (see setting discussion in sections

1.4.3 and 1.4.4) to marks from non-resident rats may suggest whether the rate of urine marking is used as a status badge (e.g. perhaps such stimuli elicit a high rate of urine marking only in dominant males). Similarly, assessing the counter-marking response of individuals to a high rate of marking from both members of their own colony and outsiders would provide interesting information on the role urine plays in communication between competitors (e.g. Hurst, 1993). Removing the dominant male and monitoring the urine marking rate of individuals occupying the ranks below the dominant from the moment after removal of the dominant until a new dominant arises could show whether subdominants repress their urine marking rate to prevent challenging the top ranking male and how urine marking changes with the establishment of a new dominant.

Urine marking and sexual communication. Assessment of any discrimination in marking and investigating by wild males in response to urine from oestrous or non oestrous females may help to show whether resident males regard unfamiliar oestrous females as potential mates or intruders (especially if social behaviour responses are also studied). A greater investigation and marking towards oestrous females would suggest that urine marking is involved in sexual communication, whereas equal marking and investigation of both females may suggest that it is involved in communication between competitors (although, under this second case there is also the possibility that males consider oestrous females as mates and non-oestrous ones as competitors). Relating the urine marking rate of isolated males kept in large enclosures with their body weight and relative dominance (conducted in experiments acceptable from an ethical point of view) and conducting choice tests of their urine

marks with receptive female wild rats may show the role urine marking plays in mate assessment. For example, receptive females may to investigate urine cues from heavy or aggressive unfamiliar males for longer than those of less aggressive males, whereas non receptive females may not show any difference in investigation or investigate for longer individuals of low status and poor fighting abilities (if they behave as competitors rather than as individuals seeking mates).

Urine marking and orientation. An important area for assessing the role that urine marking plays in orientation would be to study the responses of rats to new objects both marked and unmarked with residents' urine. These tests would show whether urine reduces neophobia. Because scent marks decay with time, they seem to have considerable potential for providing information on the time since a place or object was last visited. An example of a possible experiment may be to introduce tiles urine marked overnight and left to age for different periods of time outside the pen. Assessing the response of rats towards urine marks of different age may give interesting information about the role that scent ageing plays in the rat communication system.

Suggestions on methodology for experiments. One of the important conclusions that can be drawn from my study regards the methodology to follow when conducting similar experiments. The marks I deposited on tiles seem to have been far less successful than marks deposited by the rats in triggering a response (both urine and faecal marking and also investigatory one). Although sometimes it is impracticable to conduct an experiment using marks deposited by the rats, these type of marks should be used as stimuli whenever possible. For example, alternative designs where natural marks are

used might be achieved using laboratory rats as donors instead of wild individuals. Wild rats are very easily stressed and may not be suitable to be donors in some circumstances (e.g., if it is necessary to keep them in isolation cages). Caution concerning the use of artificial marks cannot be overemphasized on the basis of my results.

Experiments aimed at elucidating the differences between natural and artificial marks are likely to provide greater understanding of the information used by the rats. These experiments may show which semiochemicals are involved in olfactory communication and the mechanism involved. For example, urine may be only the precursor of the active compound (if this is actually produced by bacterial breakdown of urine). Alternatively, scents from sources other than urine (e.g. secretions from the food pad glands) may be inducing the marking response observed.

A further suggestion on methodology concerns the size of the arenas used. Keeping conditions as natural as possible will make results more representative of the behaviour of the species in the wild, particularly if the distribution of marks is important.

Finally, considerations of enclosure hygiene should be a compromise with the aim of the study. Enclosures should be left undisrupted by cleaning, even though infections or ammonia levels could be increased by not changing the cage bedding material or not eliminating faeces. If the pattern of urine or faecal marking plays a role in the rat communication system as these experiments indicate, disrupting this may have unknown consequences for any experiments being conducted. Even the social behaviour of the rats may be altered.

Suggestions for rodent control. Although this thesis does not belong to the field of applied research, the conclusions might have implications for rat control.

Rats appeared to prefer corners and paths by the walls of the enclosures. These may constitute the best locations for traps and poisons, particularly corners, where rats spent more time compared to open areas or sites by enclosure walls.

Latrines constitute meeting points for rats. If some kind of contact poison or pathogen had to be delivered effectively, these might be best placed in latrine sites. However, the large colony of rats studied abandoned some latrines. A similar process may occur if rats could associate a particular latrine with either their own intoxication or that of a conspecific.

Faecal marking might constitute a high level of threat to intruder rats. Chemicals involved in communication among competitors might be extracted and identified for use as a deterrent against rats. However, it is possible that these chemicals might deter competitors of poor fighting abilities but not those having high fighting abilities. In addition, if such chemicals are correlated with high fighting abilities (and probably with great resource holding potential), these chemicals may attract females seeking good mates.

Rats seem to maintain a background of urine marks which seems to be used in communication among competitors. Chemicals may be extracted and identified for use as a rat deterrent. The experiment presenting rats with clean areas showed that these contrast greatly against the urine background. Therefore, if a clean area or object carries a poison bait, it is likely that rats will recognise this as new food and avoid ingestion (Domjan, 1977; Shepherd and

Inglis, 1987). However, adding urinary chemicals to poisons and traps might increase the ingestion rate or trapping rate.

The list of possible experiments is long but achieving these would provide more understanding of an animal that is, for better or worse, so closely associated to humans.

General relevance of this research to communication.

The importance of the context in which scents are deposited and found probably constitutes the most significative contribution of the present research to the field of communication. Most published research has tested individuals in an unfamiliar clean laboratory environment, using within a cage and using scents applied by researchers and not the animals themselves. Such an approach ignores the fact that the the meaning of a signal may depend on the context in which it is deposited and found, as discussed by Inglis and Shepherd (1990). For example, if a scent is deposited as part of an individual's own territory, and later transferred to another's territory, the response of the owner of the second territory might be aggressive even if the scent marks belonged to a receptive isolated female.

In addition, it is also important to review and consider carefully which stimuli are used as controls. Blank stimuli, devoid of scents from the animals being tested have traditionally been used as controls. Such controls do not contrast against the background of a clean cage. However, in my experiments or any conducted against a normal olfactory background, a clean object may constitute a powerful stimulus and would not be a suitable control since this would contrast against a background of familiar odours. For this reason, in most experiments reported in this thesis, a control consisting of fresh familiar urine marks was compared to the

experimental stimuli consisting of fresh urine marks from unfamiliar rats.

CONCLUSIONS

The research reported in this thesis has stimulated as many or more questions as it has answered. However, this appears to be inevitable in scientific research, especially within a relatively new area such as that of rat olfactory communication. Nevertheless, some conclusions can be drawn from the studies reported here. These are the following:

i) Wild rats form latrines. The experiments showed that rats created faecal marking posts, particularly at corners, which were not a by-product of their activity. This suggests that latrines have a special function, perhaps in communication. Rats did not leave more faeces wherever they stayed longest while outside the nest, because the feeding areas appeared almost devoid of faeces, whereas the latrines held most of them.

ii) Rats could discriminate among faeces from different individuals. While we cannot be sure what features rats used to discriminate among faeces, it is very likely that olfactory cues were used in this process.

iii) Rats faecal marked in response to urine marks deposited by rats from other colonies. They did not faecal mark in response to non-social novelty, failing to faecal mark in response to clean tiles. Social novelty appeared to be necessary to trigger faecal marking. The results also showed that individual differences in faecal marking could not be attributed to body weight differences between the defecating individuals, because males of similar weights differed greatly in their faecal marking rate, while others that differed greatly in weight had similar faecal marking rates. Faecal marking did not correlate with the time spent investigating non-resident marks. It

was aimed at specific stimuli, females only faecal marking in response to female and not to male olfactory cues.

iv) Faecal marking may play a role in communication between competitors. The responses towards male and female urine cues suggest that faecal marking was aimed at competitors. Wild rats investigated and faecal marked urine marks from individuals of their own sex more than those belonging to the opposite sex (although this was less marked in the case of males). These results may be related to the context in which the marks were deposited (range marks) and does not exclude a role for faecal marking in sexual communication. For example, the relatively high faecal marking rate and investigation of wild male rats towards female marks (compared to the low female response towards male marks) might have been sexually motivated.

v) In agreement with previous reports, rats produce an uneven pattern of urine marks. Rats marked some areas more than others (which they also appeared to use more). Marking was more abundant around nesting areas and along paths by the walls than in the centre of the pen.

vi) Rats use information encoded in the distribution pattern of urine marks. They responded to gaps in the odour profile by increased marking until the density of marks was similar to that of surrounding areas. This suggests that rats use the pattern of urine distribution or the information encoded in marks for some purpose, which might be for orientation, social communication or both.

vii) Males urine mark more than females. The rate of urine marking by males was much greater than that by females, confirming some reports involving laboratory rats but contrasting with others. Results suggest that this difference in urine marking

could not be attributed solely to differences in weight between males and females. Nevertheless, a greater rate of urine marking by males compared to females does not necessarily mean that urine marking in males plays a role in communication.

viii) Rats urine mark in response to social stimuli (assuming that urine was the stimulus eliciting counter-marking). Rats urine marked olfactory cues deposited by rats from other colonies at twice the rate that they marked their own urine marks. These results, together with the longer time shown when investigating urine cues from individuals of their own sex suggest that urine marking was used in communication between competitors in the context of the experiments reported here, although the results are not conclusive.

ix) Scent marks deposited by the rats themselves appear to be more effective in eliciting responses than those applied artificially. This finding may have very important consequences for the interpretation of existing information on rat responses to odour cues. However, the factors causing this difference in response, as yet, are unknown.

x) The context in which scents are deposited and found may have a strong influence on the response of the animals being tested. For example, in the experiment examining the response of resident rats to tiles marked by other colonies, the fact that the marks serving as the stimulus were deposited by other rats as part of their territory may have triggered an aggressive scent marking response when such territorial marks from 'intruders' were found in the residents' territory. This principle needs to be considered carefully when designing experiments.

APPENDIX A.1

EXPERIMENTS ON FAECAL MARKING NOT DEVELOPED.

A.1. Differences between males and females in deposition of faeces at latrines and elsewhere.

A.1.1. Aims.

One of the characteristics of scent marking is that it is sexually dimorphic (Thiessen and Rice, 1976). Thus, monitoring the faecal deposition rates in males and females should give a clue to the role *faeces* play in social communication. More specifically, the role *latrines* play in social communication may be suggested by contrasting the sex bias in faecal deposition at latrines with that found at non latrine sites.

A.1.2. Methods.

The test consisted of two stages: In the first one food baits would be given to males and females caged individually with different dyes for each sex. In the second, the individuals would be released into a pen and the proportion of male and female faeces would be assessed at latrines (had they formed) and elsewhere.

The bait was prepared modifying slightly the protocol described by Cox (1991) in wild rats. Faeces would be dyed in red or blue using, respectively, Rhodamine B and Chicago Sky Blue. Rhodamine B was ordered from Sigma chemicals Chicago Sky Blue from Aldrich Co. The dye was dissolved in a solution 1% weight/volume in water. From this solution, 0.25 ml were extracted to apply over 500 g of wheat (0.05% weight/weight). The protocol was modified here diluting the 0.25 ml of dye in 100 ml of water to

distribute it evenly over the bait. The wheat was left overnight to dry.

The bait was offered to rats caged individually prior to their release. Males were offered wheat dyed with Chicago Sky Blue and females had Rhodamine B dyed bait. Individuals were left three days in their cages to allow them to ingest the bait.

A.1.3. Results.

Unfortunately the rats were very reluctant to eat the bait. Feeders appeared to have the same amount of food every day, some of them were spilled onto the floor of the cage. The faeces were not dyed by the end of the third day and the rats were released. No faeces found in the enclosure were dyed either, and the experiment was discarded from subsequent colonies. However, it may prove successful if individuals were habituated to the bait over a long period (several weeks).

The experiment could be extended in larger colonies distinguishing faeces from the dominant male from the rest of the members of the colony (though some way of standardising the individual contributions should be established), or, alternatively, faeces from the dominant male, the dominant female and the subordinate individuals could each be dyed in a different colour. Thus, the contribution of each social class to latrines could be assessed and hence, it would be possible to discern whether latrines constitute signposts for dominance advertising or they play other roles in social communication.

APPENDIX A.2

MISCELLANEA OF OBSERVATIONS.

Some behavioural patterns observed in the large colony seem to be similar to those reported in other burrowing colonies. Blanchard, Blanchard and Flannelly (1985) found that individuals of low social rank, which usually died later in the study, were not found sleeping in association with the dominant male. Calhoun (1962) reported that these individuals, which he called social outcasts (here referred as socially displaced individuals), used to shift their period of activity towards the daytime, apparently to avoid other rats. In my large colony of rats, at the end of the period of study, some rats were found sleeping out of the shelter during the day. Occasionally, some rats were seen eating and moving around the enclosure during the day. This behaviour seems to fit the description of the socially displaced individuals found by the authors above, but no attempt to identify the individuals was made. Therefore, it is not known whether these were socially displaced individuals of very low rank or whether the observations reflected a degree of diurnal activity by socially tolerated individuals of higher rank.

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