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Olfactory environmental enrichment of felids and the potential uses of conspecific odours

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Thesis Abstract

The potential of olfactory stimulation as a tool for the environmental enrichment of captive felids was investigated at Orana Wildlife Park in Christchurch. Six cheetah (*Acyninox jubatus*), two serval (*Felis serval*) and one tiger (*Panthera tigris*) were given various scents: male domestic cat urine; a synthetic analogue of domestic cat facial pheromone; mouse odour; peppermint and catnip, in order to determine whether scent as an environmental enrichment can effectively modify felid behaviour. All of the scents elicited a response that was significantly different to the control presentation. The synthetic feline facial pheromone elicited the greatest response, particularly from the females in the study. However, despite these results, the interest shown in the scents was limited, and due to the small sample size and other constrictions that arise from working with a zoo, the effectiveness of scent as a tool for environmental enrichment remains inconclusive and further research is needed.

The further possibilities of scent as an environmental technique were investigated at Massey University's Feline Nutrition Unit. Anoestrous and oestrous female domestic cats (*Felis catus*) were presented the urine collected form four entire male domestic cats. The social dominance ranking between the four males and the additive relationship between the males and the females in the study was established. Females were presented with different combinations of the male urine in an observation room and their behaviour recorded. The latency to approach each urine sample, the duration of sniffing, the number of flehmen responses and the number of visits to each sample were recorded as measures female interest in the urine samples.

The overall level of responsiveness appears to be quite similar during anoestrous and oestrous. During anoestrous females will investigate urine samples, however they do not appear to discriminate between the urine of different males. In oestrous the female response appears to be much more selective. A strong effect of relatedness was found for oestrous females investigating the urine of a related male. The higher the degree of relatedness to the male the lower the interest shown by the oestrous female. The dominant male also appeared to be preferred overall, and the most subordinate male preferred least overall. The dominance hierarchy could not be replicated in this study and any effect shown for dominance rank may potentially be the result of some other characteristic unique to that male. In terms of environmental enrichment potential, the time spent investigating the urine patches was limited, however the fact that oestrous females show different levels of interest in response to the urine of different males suggests that conspecific urine holds information of interest and may be useful as an enrichment tool.

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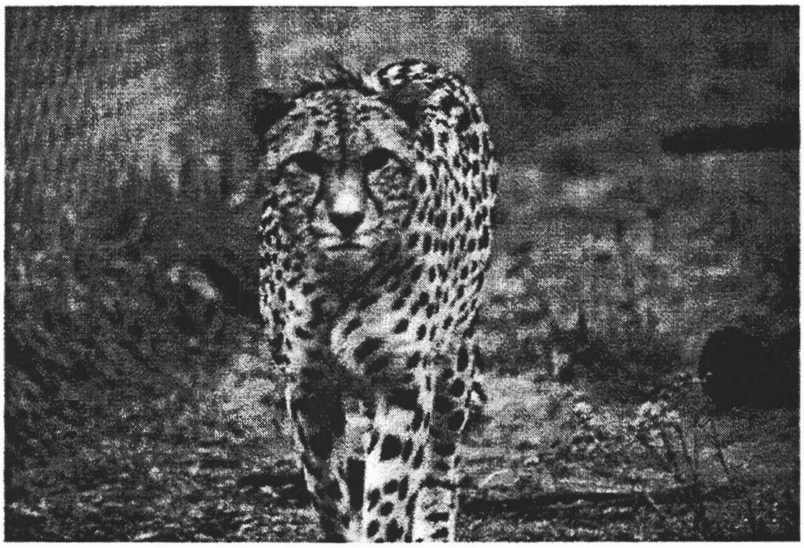
Chapter One

Environmental Enrichment



Cheetah male Kaitaia

"Similarly, all of the things which dogs, horses and monkey are made to do are merely expressions of their fear, their hope, or their joy; and consequently, they can do these things without any thought."

Descartes

1.1 Introduction to Environmental Enrichment

Environmental enrichment is a relatively new concept in the areas of animal husbandry, behaviour and welfare. The applications of environmental enrichment are as varied as the definitions and both are rife with controversy. Attention to the merits of environmental enrichment has coincided with an increased public awareness of animal welfare issues. There is a growing consensus in Western society that people have moral obligations towards the animals dependent on humans as caretakers. Consumer concern regarding the living conditions of production animals has placed pressure on industry to seek practical solutions to the problems associated with keeping animals in captivity (Anonymous, 1996). Conservation and the motivation to improve captive breeding of endangered species with the ultimate goal of releasing these animals back into their natural habitat (Newberry, 1995) has fostered interest in funding and research into the success and benefits of environmental enrichment techniques.

The theoretical aspects of this discipline have been much slower to develop compared to the practical applications of environmental enrichment. There are few operational definitions of environmental enrichment despite growing interest in this area. The use of the term environmental enrichment is often relative to the initial state of the environment in these situations, with no absolute as to what is considered an enriched environment and what is not (Shepherdson, 1998).

Controversy regarding what is considered enrichment is ongoing. Environmental enrichment is often used without a specific definition simply to describe an increase in the complexity of an animal's environment (Shepherdson, 1998). Whether this increase in complexity actually confers a benefit to the animals concerned may or may not be considered. Often environmental enrichment may be deemed successful when the benefits are aimed at meeting human demands. Such demands may include increased productivity of farm animals or increasing visitor acceptability of enclosures in zoos. In these situations any benefits to the animal's wellbeing are not always qualified. However, most commonly, environmental enrichment refers to changes to an animal's environment intended to result in an improvement in the animal's wellbeing.

Newberry (1995) defined environmental enrichment as "an improvement in the biological functioning of captive animals resulting from modifications to their environment. Evidence of improved biological functioning could include increased lifetime reproductive success, increased inclusive fitness or a correlate of these such as improved health".

Shepherdson (1998) describes environmental enrichment as an "animal husbandry principle that seeks to enhance the quality of captive animal care by identifying and providing the environmental stimuli necessary for optimal psychological and physiological wellbeing".

Although Newberry's definition may be the most precise definition with its focus on actual improvements to the animal's wellbeing via changes to its environment, Shepherdson's 1998 definition may be more practical considering the wide range of situations and intentions that have already been described as environmental enrichment. This definition is somewhat less exacting while still focusing on the improvement of the welfare of the animals concerned. Shepherdson's definition also acknowledges the process involved in attaining benefits to the animals' biological functioning, including the identification of appropriate environmental stimuli and the intention to improve wellbeing. However this does not undermine the importance of investigating the effects on animals of environmental enrichment techniques. Benefits must be proven if the use of time and money and improvements to animal wellbeing are to be maximized (Chamove, 1994).

Often definitions raise more questions than they clarify regarding environmental enrichment. It has been suggested that environmental enrichment should decrease abnormal behaviour, increase the behavioral repertoire and encourage more normal behaviour where "normal" is considered as behaviour patterns within the normal range of wild conspecifics (Chamove, 1989; Chamove and Anderson, 1989).

Yet "normal" may vary greatly in the wild, so which normal behaviours are desirable, and are some more desirable than others? Furthermore are behaviour patterns which are normal and beneficial to animals in the wild necessarily beneficial to animals that will spend all their lives in captivity? For example, the level of aggression that infant monkeys receive during infancy determines their level of aggression in adulthood (Chamove, 1980). Peer-raised individuals are less aggressive than "normally" mother-raised monkeys and will form socially harmonious groups. In many captive situations compatible social groups may be more desirable than normally aggressive ones (Chamove, 1994).

Many enrichment techniques require animals to perform behaviours that are unnatural, such as rhesus monkeys pressing levers to obtain rewards, or chimpanzees watching TV (Bloomsmith et al., 1990; Harris et al., 1999), yet improvements to their welfare are the end result. There is also evidence that although some aspects of welfare may improve, abnormal behaviour such as stereotypies, may still persist (Mason 1991). Stereotypies are repetitive, unvarying behaviour patterns with no obvious function that are common in animals living in environments that do not meet the animals needs (Mason, 1991).

Poole (1998) prescribes attributes of environmental enrichment which animals require, specifically "security, relevant complexity, opportunities for achievement and some novelty". Furthermore he suggests that environmental enrichment must also be applicable to the sensory world of the animal and to the quantity of information required to survive in the wild. How does one determine how much information is required to survive in the wild and if one could would it necessarily be the optimum for physiological and psychological wellbeing in captivity? Would institutions want or be able to provide that level of information and how can achievement be assessed? Poole does raise an important consideration however; environmental enrichment must be relevant to the species under consideration.

Enrichment techniques may be of benefit to one species and yet not to another living in a similar environment. An enrichment project for two white-sided dolphins

(*Lagenorhychus obliquidens*) and three harbor seals (*Phoca vitulina richardii*) used different stimuli such as fish, toys, tactile stimulation by humans, activation of a water jet and sounds (Markowitz and Aday, 1998). Behavioural measures indicated that the dolphins displayed a significant increase in wellbeing throughout the time that the stimuli were present. The behaviour of the seals however showed no significant change (Markowitz and Aday, 1998).

Environmental enrichment techniques are used in a wide variety of situations and species. Each situation will have its own motivations, requirements and restrictions. Each species will have its own behavioural needs to be met, each individual will have its own character and unique past experiences to take into consideration (Mason, 1991).

1.2 Environmental Enrichment Considerations

In response to public awareness of animal welfare considerations the Farm Animal Welfare Council formulated the 'five freedoms', including '(1) Freedom from pain and distress. (2) Freedom form thirst, hunger and malnutrition. (3) Freedom from injury and disease. (4) Freedom to express social and other behaviours which are rewarding for the individual animal and others, and (5) freedom from fear' (Ryder, 1996). While the first three 'freedoms' concern themselves with the physical aspect and can be met in a straightforward manner, the fourth and fifth concern themselves also with the psychological and behavioural wellbeing of the animal which is harder to define and provide for. These last two 'freedoms' enter into the domain of environmental enrichment providing the goals of improving the psychological and physical welfare of animals in captivity.

Several considerations have emerged repeatedly as important aspects of effective environmental enrichment (Carlstead and Shepherdson, 1994; Chamove, 1989; Poole, 1998; Sambrook and Buchanan-Smith, 1997). Together these components form the backbone of environmental enrichment theory. These considerations can be generally categorized as control, complexity, stress, and relevance.

1.21 Control

It is now widely accepted (Carlstead and Shepherdson, 1994; Chamove, 1989) that control over their environment is important to the psychological and physiological health of animals in captivity. An individual's control over its own actions and its environment is severely reduced within the captive situation. In the wild, animals must make their own decisions and put them into action. Many of these actions will have a direct or indirect effect on their ability to survive. In captive situations, much of an animal's control over itself and its environment is removed and placed in the hands of their human caretakers. An animal's control is particularly reduced in cages, especially regarding approach and withdrawal responses; and often in regard to merely having the opportunity to make relevant and effective motor responses (Chamove, 1989). For wild animals, the ability to withdraw from stressful situations means that the period of arousal is usually brief. In captivity, animals are usually in situations where the ability to effectively withdraw from stessors is no longer under their control. Alternative options, which are generally available to wild animals even in persistent stressful situations, are often not available in captivity.

Joffe et al. (1973) reared rats in a situation in which they had control over their environment (lighting and the presentation of food) by pressing a lever. These rats were compared to rats raised in a control group which had an identical cage set-up, but in which the timing of changes in their environment was determined by the lever pressing of the first group. Although both groups received the same amount of food and light, rats raised with control over their environment were more active and explored more than rats raised in an environment over which they had no control. Similarly, young rhesus monkeys (*Macaca mulatta*) which had control over accessing food and water were shown to exhibit greater exploratory behaviour and less self-directed behaviour than monkeys that were given the same food and water randomly (Mineka et al. 1986).

These studies suggest that in order to raise behaviourally competent animals, they should be able to produce relevant motor responses to their environment. Animals can learn that modification of their behaviour can produce a wanted change in the stimulus they are experiencing. Carlstead and Shepherdson (1994) describe ‘control’ as a psychological construct “meaning that the animal has developed contingencies between performance of a behaviour and its functional outcome”. The result from enforced loss of control often manifests itself in symptoms representing chronically high stress levels such as stereotypical behaviours (Mason, 1991).

1.22 Complexity

In this review complexity will refer to the spatial and temporal aspects of an environment in terms of its physical attributes, and in terms of novelty as a temporal component of complexity. The complexity of a captive environment can have profound long-term effects on developing animals (Carlstead and Shepherdson, 1994). A complex environment may affect changes in animals from their behaviour through to their physiology and brain development. An experimental study compared laboratory rats raised alone in small cages with no novel stimulation to laboratory rats raised in enriched environments consisting of social groups kept together in large cages exposed to novel objects and new environments on a daily basis. Rats raised in the enriched environment showed increased exploration and motor activity, had greater cerebral cortex weights, and greater dendritic branching in the visual cortex, than rats raised in the unenriched environment (Uphouse, 1980). Rats raised in enriched environments also exhibited greater behavioural diversity and complexity during the exploration of novel objects (Renner and Rosenzweig, 1986).

The skills that animals, raised in complex environments, acquire are particularly important when those animals are destined for release into the wild. The survival of captive born black-footed ferrets (*Mustela nigripes*) raised in two different captive environments has been compared. One environment involved spacious, naturalistic outdoor enclosures including natural burrows in which they could dig. The other was an indoor environment with artificial, pipe burrows in which they could not dig. Both groups were fed live prey. Once released ferrets raised in the more complex outdoor environment had a survival rate three times greater than that of the indoor raised ferrets (Vargas, 1993).

Early rearing experience with physically complex environments can result in adult individuals that are more capable of adapting their behaviour in an appropriate manner in response to novel situations. Animals that are raised without the opportunity to learn about the relationship between behaviour and its outcome are generally less inclined to actively investigate and learn about new situations (Carlstead and Shepherdson, 1994; Renner and Rosenzweig, 1986). While failure to respond in an active manner may not necessarily incur any disadvantage in a stimulus poor environment, it can be very important if those animals are destined for breeding, released into the wild, or have to deal with novel stressful situations (Carlstead and Shepherdson, 1994).

Most animals kept in captivity show a vastly smaller range of behaviours compared to conspecifics in the wild (Poole, 1998; Chamove, 1989). This diminished range of behaviour is often attributed to inadequate enclosure design and lack of stimulation (Chamove, 1989). It is not merely the size of the enclosure that is of importance rather it is the quality of the facility it offers, which will effect the wellbeing of its inmates (Mellen, 1991; Poole, 1998). Studies have shown that increasing the range of foods eaten is healthy, increasing the range of muscles used is healthier and increasing the range of experiences to which an animal is exposed further improves the health benefits and is more stimulating (Chamove, 1989). All of these are aspects of potential enrichment approaches that can stimulate a wider range of behaviours.

1.23 Stress

The reduction of stress is a common aim of many enrichment studies, yet the very nature and application of the term stress is still an area of ongoing controversy (Wemelsfelder, 1984). Some authors argue that using the term 'stress' has an inherently negative connotation, others argue that 'stress' is, or should be, a biologically neutral concept, unrelated to its everyday social associations of stress as distress (Chamove, 1989). Stress is often described as a continuum ranging from the 'stress' effect of under-stimulation (Wemelsfelder, 1984), to the beneficial effects of acutely stressful stimulation (Moodie and Chamove, 1990), through to the stress of physiological and psychological exhaustion resulting from over stimulation (Wemelsfelder, 1984).

Garmezy (1982) defines stress as "any action or situation that places special physical or psychological demands upon a person- anything that serves to unbalance an individual's equilibrium or homeostasis". With this definition of stress, beneficial or welfare enhancing events may also be described as stressful. As discussed above novel and complex environments and events may be stressful yet they produce individuals better able to cope in future stressful situations. This indicates that some stress is desirable, and a lack of stress may lead to under-stimulation causing the animals to be excessively lethargic and disinterested. When considering environmental enrichment stress can be largely equated with arousal. Indeed, 'arousal' is often used as a neutral term, to describe any "stressful" events that demand effort from the animal (Chamove, 1989; Carlstead and Shepherdson, 2000). 'Stress' frequently refers to the long-term repercussions of negative incidences. It has been suggested that rather than trying to remove stress from the captive environment, the more relevant goal may be to provide an optimal level of stress (Chamove, 1989).

In the wild, animals face the varying challenges of surviving and providing for themselves. Perhaps the critical factor between the stresses faced by captive animals and those faced by wild animals is that non-lethal stresses in the wild tend to be of a brief duration whereas those in captivity tend to be persistent or repetitive. Some situations, which general consensus would agree are stressful, may in fact produce beneficial effects. Moodie and Chamove (1990) found that a brief threatening event, such as a hawk-shaped silhouette that elicited predator avoidance behaviour from captive tamarins, had beneficial effects similar to those found in successful enrichment studies.

Defining an animal's homeostatic state is often used to approach the problem of determining what is stressful to an animal by comparing it to what is "normal" for an animal.) Within this context, behaviour is viewed as the way an animal adapts to its environment and maintains homeostasis (Baxter, 1982; Bure, 1981; Wiepkema, 1982). So if an animals adapts its behaviour and returns to its homeostatic state, that state is regarded as 'normal' and no longer subject to stress. It is known that abnormal or undesirable behaviours, particularly stereotypical behaviours reduce the corticosteroid level in animals which are subject to chronic stress (Dantzer, 1981, Dantzer and Mormede, 1981). This suggests that the performance of stereotypical behaviours need not be regarded as signs of stress within themselves, but as a way of reducing stress (Wiepkema, 1982). Wemelsfelder's (1984) response to this view is that although the performance of such abnormal behaviour may reduce stress to the animal in consideration, the cost of this reduction is the distortion of the animal's intrinsic nature.

There is no one way of determining what amount of stress is optimal, and thereby allowing the animal to function at intermediate levels of arousal. Close behavioural monitoring is required to ensure each individual's response to stimulation is of an enriching nature.

1.24 Relevance

Poole (1998) states that environmental enrichment must be appropriate to the sensory world of the animal. Any methods used to enrich the environment with the aim of improving the animal's welfare must have meaning for the animal. In order to do this the desired behaviour must first be identified along with the ensuing benefit the animal will gain by performing these behaviours (Newberry, 1995), otherwise there is a risk of assuming the benefit of enrichment without any effective change in the animal's welfare.

The promotion of a broader range of behaviours, specifically those performed in the wild, is generally considered an aim of environmental enrichment (Chamove, 1989; Newberry, 1995; Poole, 1998). The creation of naturalistic environments may be particularly important for animals in a captive breeding program which are destined to return to the wild e.g. black footed ferrets (Vargas, 1993). The ability to survive in the wild will require successful recognition of food, predators and potential mates.

The greatest risk that is likely to be faced within the context of environmental enrichment is the temptation to view the situation anthropomorphically. Whether much environmental enrichment is aimed at humans and what humans perceive as being beneficial, in order to alleviate guilt and give a 'feel good' experience is a common debate in environmental enrichment. This is particularly relevant for zoos where visitors like to see the animals in green and natural environment but often the “enrichments” to enclosures may have no tangible benefits for the animals.

The behavioural needs of an animal can be very difficult to identify and ensuring that these needs are met is a challenge for those who keep them in captivity. The first question that needs to be asked is what is known about the biology of the species and the needs of closely related species (Poole 1998)? Studies on stereotypical behaviour and environmental enrichment reveal that certain species cope better with confinement and the lack of stimulation than others. Animals such as pigs, primates and carnivores frequently exhibit stereotyped behaviours (Blackshaw and McVeigh, 1984; Chamove, 1989; Mellen, 1991) whereas herbivores do so to a lesser extent (Poole, 1998). This suggests that the natural (wild) way of life may impact on how that species copes with captivity (Poole 1998). Identification of the factors in terms of the quantity and quality of the stimulation an animal receives during its life could be invaluable information used to maintain the species in captivity. Poole (1998) put forward five considerations for devising enrichment techniques or enclosure design for a particular species.

(1) Longevity. This determines the amount of information that an animal is able to acquire during its lifetime. Longer living species gather a greater range of learnt experiences, which they can take into consideration when making a decision (Moss, 1988). Longer living species may rely more on such acquired knowledge than short-lived species.
(2) Knowledge required in order to develop successful foraging techniques. Omnivores that depend on a large variety of foods and carnivores that must learn to locate and catch their prey must acquire complex skills in order to survive independently.
(3) Vulnerability to predators. This requires the ability to detect the signs of the presence of possible predators and the correct and corresponding escape techniques.
(4) Social Complexity. Living in social groups may require a greater awareness of the motivation, status and relationships of other group members.

(5) The type of terrain the species naturally lives in. The greater the complexity of the terrain the more the animal will have to know about the character of the environment.

These are all factors, which are highly relevant to the life style of a species. However it will be difficult to know how to weight them when considering behavioural needs (Poole, 1998). Further considerations must be made when considering the use of 'artificial' stimuli for enrichment purposes. The provision of inanimate objects, such as toys, must be relevant to the behaviour of the species and what may seem to make intuitive sense (e.g. Gorilla liking forest sound) may not always be the case in reality (Mench, 1998; Ogden et al., 1994).

1.3 Conspecifics as Enrichment

Companionship may be one of the most important methods of enriching the lives of many species living in captivity. Compatible companionship may offer opportunities for engaging in a wider variety of natural behaviours, which is frequently considered to represent enrichment (Chamove, 1989).

For social animals such as primate species which live in highly complex groups in the wild, supplying compatible partners may be the most effective form of enrichment, even to the point of attenuating the effects of other environmental enrichment techniques. Schapiro et al. (1997) observed that the effects of simple and complex enrichment regimes held similar influence over a group of macaques (*Macaca mulatta*). The benefits that may have been bestowed on the macaques by the more complex enrichment scheme were attenuated by the social interactions of the group. Such evidence suggests that some enrichment techniques be of less relevance to animals that are actively engaged in the social dynamics of their group and have their social needs met (Schapiro et al., 1997).

What is appropriate companionship is decided by taking account of the nature and variety of groups in the relevant species in natural conditions. Despite the fact that many species of felid are solitary, they are frequently housed together in captivity. Similarly social felids are sometimes kept individually. Housing cats together can be a source of enrichment or a source of chronic stress. Species differences are also confounded by individual differences in behaviour. Some individuals seem to be able to share an enclosure without stress yet other combinations of the same species will appear constantly fearful when residing with a conspecific (Mellen et al., 1998).

Small felids housed in groups of three spend less time pacing than those housed in pairs (Mellen, 1991). However, the overall proportion of time spent active decreased for those cats housed in trios. Individuals kept in trios were also less likely to reproduce than those kept in pairs (Mellen, 1991). Overall the data indicates that cats of a solitary nature, housed in trios, exhibit decreased activity. This decreased activity is likely to stem from social stress and involuntary proximity implying that small cats should be housed in groups no larger than two (Mellen, 1991).

Apart from the species-appropriate considerations, there may also be considerations of compatibility between individuals. This may hold greater relevance when mating pairs are considered. Although the understanding of mate selection or the principles of sexual attraction may still be rudimentary they must be considered to have an impact in captive breeding programs (Lindburg and Fitch-Snyder, 1994).

An incompatible pair of Siberian tigers (*Panthera tigris altaica*) kept at Metro Washington Park Zoo in Portland, Oregon, USA, is kept alternatively in the exhibit enclosure. While one tiger is in the exhibit enclosure the other is kept in a holding area out the back. Each tiger has access to the enclosure for half the day. On being released into the enclosure each tiger will explore the scent marking of the other tiger and add its own to the milieu (Mellen et al., 1998). Although these tigers are not housed together, they are provided with relevant social enrichment. In zoos and similar institutions this stimulation of natural behaviour also allows the public to see more active cats showing a wider variety of behaviours (Mellen et al., 1998).

1.4 Felids in Captivity

Like the constantly dynamic and challenging environment that wild felids live in, enrichment methods for felid environments in captivity must also be constantly changing and challenging. Felids, like many other organisms, will often habituate quickly to new conditions and devices to which they are frequently exposed (Mellen et al., 1998). So to create and maintain happy and well adjusted individuals in captivity, no single technique will suffice. To make environmental enrichment a viable and successful option an ongoing commitment to variety and maintenance must be made. The promotion of the greater range of behaviours seen in wild conspecifics is the motivating aim of much environmental enrichment (Chamove, 1989; Poole, 1998; Seidensticker and Fortham, 1994). An optimal environment in captivity should provide animals with the opportunity to exhibit these natural behaviours.

Wild felids range over vast areas in search of prey and breeding opportunities and use both scent marks and vocal cues to communicate with conspecifics. Enclosure spaces in zoos are always limited and enclosures are always substantially smaller than natural home ranges. Contact with conspecifics may not be possible or it may include only a small number of individuals all housed together. Genetics and demography guide breeding programs (such as species survival plans). The restrictions of these considerations, combined with the limited enclosure space available to most institutions, often mean that the breeding and rearing of offspring is under tight control.

1.41 Care-taking and Housing

Caretaker interactions that habitually involved talking to, scratching, or playing with a cat can have beneficial effects for the animals in captivity. Small cats in captivity that spent more time interacting with their keepers tended to spend less time pacing and were more likely to successfully produce offspring than animals who had only minimal contact with their care-takers (Mellen, 1991). This study suggests that cats that are confined are more affected by the manner, particularly the predictability, in which humans treat them (Mellen, 1991).

Carlstead et al. (1993b) highlighted that the quality of the caretaking can be significant to the welfare of caged domestic cats, and by implication extended this consideration to non-domestic cats. However, it may be that the predictability of the caretaking routine rather than the quality of the interaction is responsible for the greater reproductive success and lack of obvious indicators of stress observed. The data does not distinguish between these two alternatives (Carlstead et al., 1993b).

Interspecific interactions may involve proximity to natural predators. Zoos tend to group by continent or phylum. Both of these forms of organisation tend to encourage groupings such as cheetah or small cats being housed close to large *Panthera* species such as lions which the smaller cats may perceive as predators, causing chronic stress (Carlstead et al., 1993a; Wielebnowski et al., 2002).

Furthermore, many cats are often housed in groups, regardless of their natural social tendencies. Reasons for this may vary from a lack of space and funding to supply suitable housing to a lack of understanding of the animals natural biology confounded by an anthropomorphic view that animals (even those solitary in nature) will be lonely kept in an enclosure all on their own. Mellen (1991) found that small cats housed in groups greater than two were unlikely to breed and were almost never able to raise their litters.

1.42 Inactivity

A study of the time budgets of small captive felids in captivity indicated that the time spent inactive during the day was consistent with the available data on time budgets of wild counterparts (Mellen et al., 1998). The consistency between wild and captive conspecifics suggests that the amount of time spent inactive during the day is largely fixed. Therefore, attempts to increase activity during the day may be ineffective or even counterproductive (Hutchins et al. 1984). Greater enrichment success may lie in attempts to encourage felids to repartition the time they spend in activity; for example, spending more time investigating their environment and less time pacing (Mellen et al., 1998).

1.43 Pacing

Felids can be motivated to carry out feeding-related appetitive behaviours in the absence of the need to satisfy any hunger (Eaton, 1972; Hughes and Duncan, 1988; Leyhausen, 1979). A lack of opportunities to perform these behaviours can result in behaviours that are considered aberrant (Shepherdson et al., 1993). Small cats in the wild will opportunistically hunt any prey encountered while patrolling their home range, or wait in concealment to ambush prey Small felids generally hunt small prey so these behaviours are commonly repeated several times a day (Mellen et al., 1998). The type of prey, method and frequency of hunting will vary somewhat from species to species.

Although studies support the theory that pacing is related to higher stress levels, this does not mean that this hypothesis and the hypothesis that pacing is representative of appetitive behaviours are mutually exclusive. If pacing were exhibited only as a coping mechanism (Mason, 1991), a symptom of living in an inadequate environment, then pacing should not be performed by individuals living in complex environments with many hiding places. However the indications from the above studies are that pacing will still occur to some degree even in complex environment with sufficient hiding places.

Mellen et al. (1998) stated that their subjective observations supported the appetitive hypothesis, observing that pacing was easily interrupted by immediate changes in the surrounding environment, such as birds flying past or keepers walking past the enclosure. This suggests that pacing is not entirely motivated by stress. Pacing may derive from a form of searching behaviour in captive felids but the motivation of that pacing will probably vary in different captive environments. Motivation is of vital importance when considering animal welfare (Carlstead et al., 1992).

In some enclosures, pacing may be motivated by a need to search for food, mates or the patrolling of home ranges (Freeman, 1983) and may be viewed as a healthy expression of natural behaviour in a spatially restricted enclosure. In other enclosures pacing may be motivated by the desire for a place to hide or some other aspect of its needs which have not been met, and may indicate decreased well-being. Enclosure design may have a strong effect on the motivation of the pacing exhibited by felids in captivity and because the alternative motivations are unlikely to be mutually exclusive it can be difficult to determine how the motivation changes with differing enclosure designs (Mellen et al., 1998).

1.44 Environmental Complexity

Small felids spend significantly less time pacing in enclosures that are more complex environments, specifically those with more visual barriers or hiding places (Mellen et al., 1998). Visual barriers were the variable found to have the greatest effect on the time spent pacing. In enclosures containing seven or more visual barriers pacing was found to be reduced or non-existent (Mellen et al., 1998).

A similar study found that pacing in leopard cats (*Felis bengalensis*) could by reduced by up to fifty percent by providing paces to hide (Carlstead et al., 1993a). Both pacing and urinary cortisol levels decreased when places to hide were added to an enclosure. In a study of domestic cats (*Felis catus*) Carlstead et al. (1993b) found that cats that hid more often usually had lower average cortisol levels, indicating that having somewhere to hide is important for reducing stress for captive felids in an unpredictable environment. Another aspect of enclosure quality is enclosure size. Naturally the enclosure size captive felids require will vary with their size, natural lifestyle and with the number of individuals housed together. In general, complexity seems to have a greater impact on improving the welfare of animals over size without complexity (Mellen, 1991).

Together these studies indicate that more complex environments reduce stress and pacing in captive felids. Although there could be difficulties with zoos, whose aim it is to be able to constantly exhibit these animals, creative solutions must be able to be found which meet both and animals needs yet still allow the public the opportunity to view the animal.

1.5 Diet and Food Presentation for Felids

Providing enrichment opportunities for captive carnivores especially solitary felids is among the most challenging areas of environmental enrichment. Many of the problems arise from the "stalk-rush-kill" method that felids employ to acquire their food (Leyhausen, 1979). Prey is then dispatched using a suffocating bite to the trachea or a fatal bite to the back of the neck (Ewer, 1973). If we accept that, as commonly recommended, the natural behaviours of the species in the wild should be encouraged in the species in captivity (Seidensticker and Fortham, 1994), this enrichment goal causes both ethical and practical difficulties with regard to felids' method of obtaining food.

Considerations about the welfare of the prey animal and negative reactions of the public to providing live prey mean that most zoos cannot feed their cats live prey (Lindburg, 1988). However this leaves a primary behaviour pattern without a constructive outlet. Convenience and efforts to provide a nutritious diet have resulted in captive carnivore often being fed specially prepared meat diets, which are only vaguely reminiscent of their natural prey.

Most dietary studies have concentrated predominantly on meeting the physiological requirements of captive felids (Lindburg, 1988). Duckler and Binder (1997) found unusual muscular attachment in the head and jaw area of large felids that had spent all their lives in zoos. These deformities are the result of unnatural diets and excessive grooming, symptomatic of the captive environments.

The principal muscles operating the jaw and neck area which are used for killing, biting, holding and shaking prey appeared to be much less developed in captive animals causing changes in the shape of the skull (Duckler 1998). More recent research has paid attention to the non-nutritional components of diet (Bond and Lindburg, 1990; Lindburg, 1988; McPhee, 2002). Animals that were exclusively fed a prepared diet often developed tooth decay, dental pathologies, muscle atrophy and generally poor health despite their diet being nutritionally balanced (Bond and Lindburg, 1990).

1.51 Whole Carcasses

The obvious and frequently applied solution to this problem is to provide humanely killed whole carcasses or carcass fragments. Choice of 'prey' species can vary from rats and mice for smaller felids through to whole goats for the large species (Mellen et al., 1998). When felids are provided with entire carcasses they may demonstrate the whole or partial stalk-rush-kill sequence (Lindburg, 1998). Feeding whole carcasses has a well-documented physical health advantage and may also provide psychological benefits to animals frustrated at not being able to perform appetitive behaviours.

The presentation of carcasses may enhance psychological and physical wellbeing in felids (Bond and Lindburg, 1990; Hutchins et al., 1984; McPhee, 2002). Feeding on carcasses provides a novel experience for felids living in a largely invariant captive environment. Whole or partial carcasses fed to captive felids may assist in breaking the monotony of their daily routine and improve health (Bond and Lindburg, 1990; Hutchins et al., 1984). Carcass-fed cheetahs showed larger appetites, spent more time feeding and were more possessive of food compared with cheetahs on a commercial diet (Bond and Lindburg, 1990). The greater amount of time involved in feeding on carcasses was due to more time spent approaching and smelling the food, and more time spent chewing and using molars to slice food.

Lindburg (1988) found that whole carcasses fed to cheetah often sparked bouts of play, and interpreted the observed changes in behaviour as indicative of improved wellbeing. If pacing is motivated by appetitive behaviour then the changes in behaviour seen in these studies could indicate that felids may be encouraged to replace time spent pacing with more time spent processing food by feeding them carcasses.

1.52 Hiding Food

Hiding food and multiple feedings can avert monotony and predictability and can stimulate hunting and foraging behaviour in many mammals (Carlstead et al. 1991). Shepherdson et al (1993) found that multiple feedings hidden in small brush piles increased the exploratory behaviour of leopard cats and also increased the range of behaviour the cats exhibited. Law (1993) found that hiding dried fish in a log-pile had similar results for a pair of jaguars. Large pieces of meat suspended from the top of the enclosure had similar success, stimulating hunting and leaping behaviour (Law 1993). In general modes of presentation which encourage felids to use effort to hunt and search out their food are favourable methods to encourage activity and enrich the environment of captive felids.

1.53 Live Prey

Although the provision of live mammals and birds is often illegal, due to their own welfare considerations, and undesirable in public perception, the provision of live fish to appropriate species has proved a very effective enrichment technique. Shepherdson et al. (1993) found that fishing cats that were provided with live fish in a small pond exhibited a sixty percent reduction in time spent sleeping and an greater use of enclosure space. Other felids such as tigers (*Panthera tigris*), jaguars (*P. onca*), and ocelot (*Leopardus pardalis*) have also shown hunting behaviours when presented with live fish (Mellen et al., 1998). For smaller felids, live insects such as crickets provide alternative possibilities for live prey (Mellen et al., 1998).

1.54 Simulated Hunt

Various mechanical devices have been used to exercise and entertain carnivores in captivity. At the Duisburg Zoo, Germany, a dummy zebra baited with meat on a cable and pulley system was installed in the Cape hunting dogs' enclosure (Gewalt 1992). A similar device was installed in a serval enclosure at Washington Park Zoo, Portland, USA. The aim of the device was to encourage a pair of servals to use their 'special leaping abilities' (Mellen et al., 1981). The device was particularly successful with the female who perform a mean of 94.4 jumps per hour while the machine was active, while the male showed no significant change in his behaviour. The variation in the success of this particular attempt was attributed to idiosyncratic differences in the individuals (Mellen et al., 1981). Such device devices have also been used for cheetah (Williams et al., 1996) resulting in an increase in running and time spent observing their surroundings by the cheetah.

Mimicking the contingencies of the hunt in captivity can be very difficult (Markowitz, 1995). A computer controlled acoustic prey device used speakers to simulate sounds of a bird flying across the enclosure of a singly housed adult leopard's enclosure, in an attempt to engage the animal in a wider rang of hunting behaviours (Markowitz, 1995). When the leopard had successfully tracked the 'prey', motion sensors activated the delivery of a food treat. Stereotypical behaviours decreased significantly and the leopard showed a significant increase in activity and a wider range of species-typical behaviours (Markowitz, 1995).

The techniques used to stimulate hunting behaviour are commonly found to be effective (Carlstead, 1991; Lindburg, 1988; Markowitz, 1995; Mellen et al., 1998; Shepherdson, 1993). Although nutrition is not the primary motive, the use of food is an effective means of encouraging participation and ensuring the general success of the enrichment technique (Carlstead, 1991).

1.6 Reproduction

Reproduction is an important aspect in any consideration of captive animals. The state of animal welfare in the captive environment has a duel interaction with reproduction. In the case of production farm animals (Okere and Nelson, 2002), or zoo animals that are a vital link in captive breeding programs (Freeman, 1983), successful reproduction can be very important and highly desirable. The perceived state of the environment, the degree to which the animal's needs are met may determine the ease with which reproduction is accomplished (Carlstead and Seidensticker, 1991). The state of welfare can determine the success of reproduction (Mellen et al., 1991). Alternatively reproduction may determine the state of welfare. The whole process from mate choice through to weaning and separation of mother and offspring provides a wealth of stimulation, learning and achievement opportunities (Lindburg and Fitch-Snyder 1994). It can facilitate a wide range of natural behaviours, the often-cited goal of environmental enrichment (Chamove, 1989; Newberry, 1995; Poole, 1998; Seidensticker and Fortham, 1994). It also facilitates a positive functional outcome to a host of activities which animals are instinctively motivated to perform (Hughes and Duncan, 1988). Territory patrolling in search of potential mates can lead to frustration and stereotypical pacing in captive carnivores (Carlstead and Seidensticker, 1991). This infers that introduction to prospective mates and successful copulation may provide a welfare enhancing perception of functional outcomes as a result of a set of behaviours.

1.61 Impact of Stress on Reproduction

On-going stress can have serious effects in terms of reproduction function. The hypothalamic-pituitary-adrenal axis produce stress hormones that may result in a reduction of steroid hormone levels. Such changes can cause the suppression of reproductive physiological function (Carlstead and Shepherdson, 1994). In socially living male olive baboons (*Papio anubis*) stress-related increases in glucocorticoid secretion were found to act directly at the testicular level (Sapolsky, 1987). Low social ranking in marmosets (*Saguinus fuscicollis*) under social stress from overcrowding is associated with hormonal suppression of fertility (Epple, 1978). The hormonally mediated development of offspring and their sexual behaviour as adults can all be affected by stress experienced by the mother while her progeny are still in utero. Stresses resulting from husbandry procedures such as relocation or removal from the social group during pregnancy can affect the viability of the offspring. Ward (1972) found that female rats stressed daily in their last week of gestation gave birth to male rats that exhibited a decreased rate of sexual behaviour as adults.

Conversely, although chronic stress has a repressive effect on reproduction, acute stress may be associated with sexual activation. There is ample evidence of species in which acute stress may promote or even be a prerequisite for reproductive activation (Antelman and Caggiula, 1980; Carter et al., 1986; Sapolsky, 1987).

These seemingly opposite effects of stress on reproduction suggest that an optimum level of stress may be of benefit. Under these circumstances stress may be better described as challenge or stimulation. An appropriate level of stimulation, such as environmental novelty or uncertainty (Carlstead and Shepherdson, 1994), can contribute to improved state of vigilance and responsiveness to changes in the environment (Danzer and Mormede 1983). As discussed earlier, protracted exposure to a stimulus-poor environment can result in apathy, low levels of exploratory behaviour and difficulty dealing with novel situations. Over-stimulation causing high levels of arousal is often associated with "fight-flight" response or behavioural suppression (Carlstead and Shepherdson, 1994). Intermediate levels of stimulation and arousal correlate with improved alertness, faster learning rates and enhanced immune response (Weiss et al., 1989). An individual animal's ability to cope with stress may determine whether its reproductive activity is stimulated or suppressed (Carlstead and Shepherdson, 1994).

1.62 Impact of Environmental Enrichment on Reproduction

In most cases evidence that environmental enrichment encourages reproduction is largely implied rather than proven experimentally (Carlstead and Shepherdson, 1994). Bigger and more complex environments which supplied refuges from the public and conspecifics were found to have a beneficial impact on the maternal behaviour of female captive gorillas increasing the chance of infants being reared normally (Miller-Schroeder and Paterson, 1989). If environmental enrichment succeeds in both positively stimulating individuals and also reducing chronic stress then the implication for reproductive success is likely to be beneficial.

There is a greater body of anecdotal evidence that environmental stimulation increases reproductive activity. Davis (1964) reported that a pair of cheetah attempted their first mating after killing live prey for the first time. Hunting live prey, translocation and stressful veterinary procedures have all reportedly been events which coincidentally facilitated breeding (Seager and Demorest, 1978; Sterns, 1991). Arousing aggressive interaction between cheetah pairs is associated with successful conception (Manton, 1970; Benzon and Smith, 1974).

Most of this evidence is the result of what Carlstead and Shepherdson (1994) term "fortuitous" enrichment, and although anecdotal, these reports support the theory that stress-inducing events of short duration can have beneficial effects on welfare and reproduction. Of course reactions to acute stress will vary between species and between individuals and must be carefully monitored and assessed before becoming incorporated as part of an environmental enrichment program to improve breeding.

1.63 Mate Choice

Reproductive failure in captive pairs of animals frequently leads to the suspicion that at least one of the pair is infertile, when the issue may be one of incompatibility with the only mate available (Lindburg and Fitch-Snyder 1994). The occurrence of mate choice may be more common than is frequently realized, and the practice of dispersing breeding animals in genetically preferred pairs leaves little room for incompatibility (Lindburg and Fitch-Snyder 1994). Certainly for polygamous animals this mate restriction runs counter to biology, yet even in monogamous species incompatibility may be the underlying cause for reproductive failure (Freeman, 1983). Correlates of breeding success need to be looked into for each species, so that educated decisions can be made on whether to form new pairs if breeding does not occur within a certain period of time and how best to group the animals. This way the optimum strategy can be formed for each survival plan (Freeman 1983).

In such a study, looking at correlates of reproductive success for captive snow leopards, Freeman (1983) found that successfully breeding pairs behaved differently from unsuccessful pairs, even in those years when they did not produce offspring. Successful animals of both sexes were more active than reproductively unsuccessful animals. They spent a greater amount of time investigating their enclosure, interacting together, grooming themselves and demonstrating the flehmen response when sniffing. Unsuccessful males were found to continue pacing when the female was in oestrus whereas an extreme drop in this behaviour was seen in successful males during the period when females are in oestrus. (Freeman, 1983).

There is little in the environmental enrichment literature considering the impact on welfare, good or bad, of successful reproduction. Certainly for some individuals living in restricted space, with genetically restricted partners, the issues of overcrowding and inbreeding may mean an overall decrease in wellbeing. Where there is ample room and the presence of genetically viable and compatible breeding partners, reproduction may be a powerful source of social enrichment, providing on-going novelty. Reproduction in mammals starts with mate location and choice, continuing through to courtship, mating, pregnancy, parturition, and the raising of offspring (Lindburg and Fitch-Snyder, 1994).

Each stage provides unique challenges and experiences. Reproduction is an important biological drive, and so relevant to all species. Sexually mature females in the wild are frequently pregnant or accompanied by their offspring (Bradshaw, 1992; Caro, 1994; Schaller, 1972).

Social species kept in groups appear to cope with the captive environment better than solitary species, particularly solitary carnivores. The experiences and interactions that are inherent with successful reproduction may be an effective way for solitary species to interact with other individuals, learn new behaviours with functional outcomes and occupy their time in a positive and productive way. All of these experiences can potentially have a vital and positive impact on the welfare of animals kept in captivity.

1.7 Objects and Odours

Other things apart from food can be used to elicit a wider range of behaviours and activity. Novel objects can induce elements of hunting behaviour, play and social interaction. Stalking, pouncing (Mellen et al. 1998) and even feline versions of football (personal observation) can be induced by the presence of boom balls. Boom balls can be cut open slightly and stuffed with bones etc to encourage interest. Animal skins also stimulated a wide range of behaviours and managed to maintain interest longer than plastic objects (Mellen et al. 1998).

Strong interest can often be elicited by novel odours. Felids leave and collect social information with conspecifics in the form of urine marks, scrapes, and claw sharpening (Mellen, 1993; Gorman and Trowbridge, 1989). Various novel scents have been used to increase and improve the olfactory world of captive felids. Hunter's commercial deer musk, spices, in particular mace, allspice, cumin, nutmeg (Powell 1995), fresh catnip, lanolin, rose petals, or faeces from prey animals (Mellen et al. 1998). Once again the presentation mode, both spatially and temporally, can be important and may determine the effect as much as the type of odour used (Mellen et al. 1998). Relatively little research has focused on the environmental enrichment potential of scent (Carlstead and

Seidensticker, 1991; Powell, 1995; Mellen et al. 1998) despite the significance of olfaction to felids (Gorman and Trowbridge, 1989).

1.8 Aims

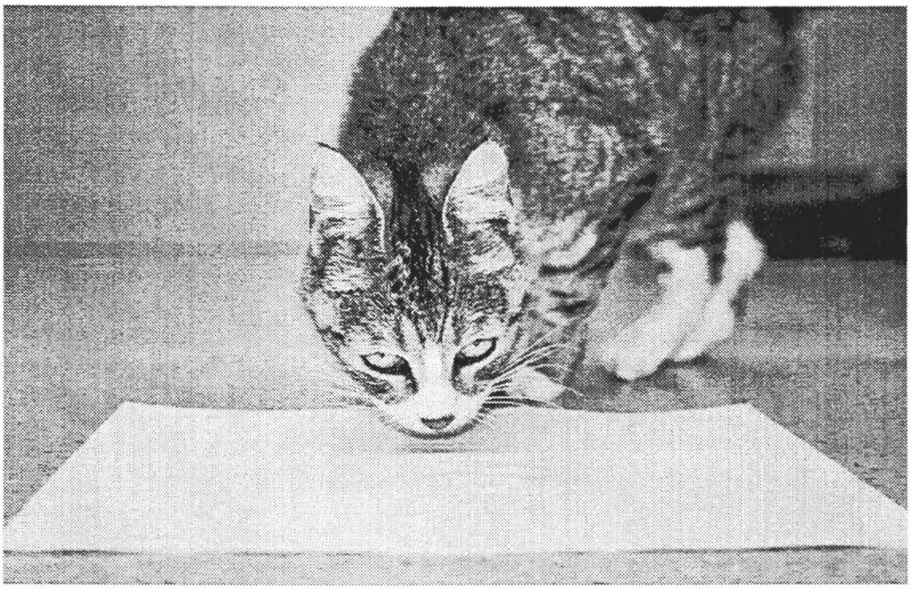
All cats are surrounded by a plethora of scents, many of which convey specific information. An animal's daily life is undoubtedly influenced by scents, a female in oestrus, a potential male mate, the track of an intruder, an old kill, or potential prey, and it is an aspect of felid lives in which there is still much to explore. The potential for olfactory environmental enrichment is likely to be great and may be currently under-utilised.

In order to investigate the potential of olfactory stimulation for environmental enrichment five difference scents were presented to different species of exotic felids kept at Orana Wildlife Park in Christchurch (see chapter four). The scents used were urine from an entire male domestic cat, peppermint extract, catnip spray, mice faeces and a synthetic analogue of domestic cat facial pheromones 'Feliway' (Sanofi Sante Nutrition Animale, France). However the small number of animals, uncontrollable environmental variance and the difficulties of co-ordinating with a zoo meant that the effectiveness of these scents as environmental enrichment tools could not be fully investigated.

A different line of enquiry was then pursued at the Heinz Watties Feline Nutrition Unit, a Massey University research unit in Palmerston North (chapters two and three). The potential environmental enrichment effects for felids, of conspecific urine, were investigated using domestic cats *Felis catus* as a model for captive wild felids. Anoestrus and oestrous female cats were presented with urine samples collected from four entire male cats. Various behavioural parameters, including latency, sniff duration, number of flehmen responses and number of visits to a scent source were recorded as measures of female interest. The level of female interest in the urine of entire males is used as an indicator of the potential of conspecific urine as an environmental enrichment tool for wild felids kept in captivity.

Chapter Two

The Response of Anoestrous Female Domestic Cats (*Felis catus*) to Conspecific Male Urine



Nikki investigates a urine sample

all good kumrads you can tell
by their altruistic smell.

e.e. cummings

Abstract

The environmental enrichment potential of conspecific urine was investigated at the Heinz Watties Feline Nutrition Unit, a Massey University research unit in Palmerston North, New Zealand. Ten anoestrus entire females were presented with urine samples collected from four entire males housed at the unit. The latency to approach, the sniff duration, number of flehmen responses and the number of visits to each urine sample were recorded as indications of female interest in the urine samples. Each male's urine sample was investigated but the female response was limited. Females did not appear to distinguish between individual male urine samples and the level of relatedness to a male had no affect on the female response. Individual females consistently expressed different levels of curiosity in response to the urine samples.

2.1 Introduction

The few studies that have investigated the benefits of various environmental enrichment techniques for domestic cats have focused on the benefits of social enrichment and the provision of novel objects for isolated laboratory cats (Hurni and Rossbach, 1986; McCune, 1995; de Monte and Le Pape, 1997). Domestic cats may be useful surrogates for captive wild felids when the effectiveness of new enrichment techniques is being researched. Wild felids held in captivity share many of the environmental inadequacies and challenges that many laboratory cats face. However research on wild felids in captivity can be restricted by small sample sizes, the challenges of co-operating with zoological institutions, and environmental variance from the uncontrollable conditions of a public facility (see chapter 4). Under these circumstances it may be difficult to accurately assess the effectiveness of a new enrichment technique. Many behavioural patterns are common to all the Felidae (Macdonald, 1980; Gorman and Trowbridge, 1989), and the responses of domestic cats may act as an indicator of the probable responses of other felids.

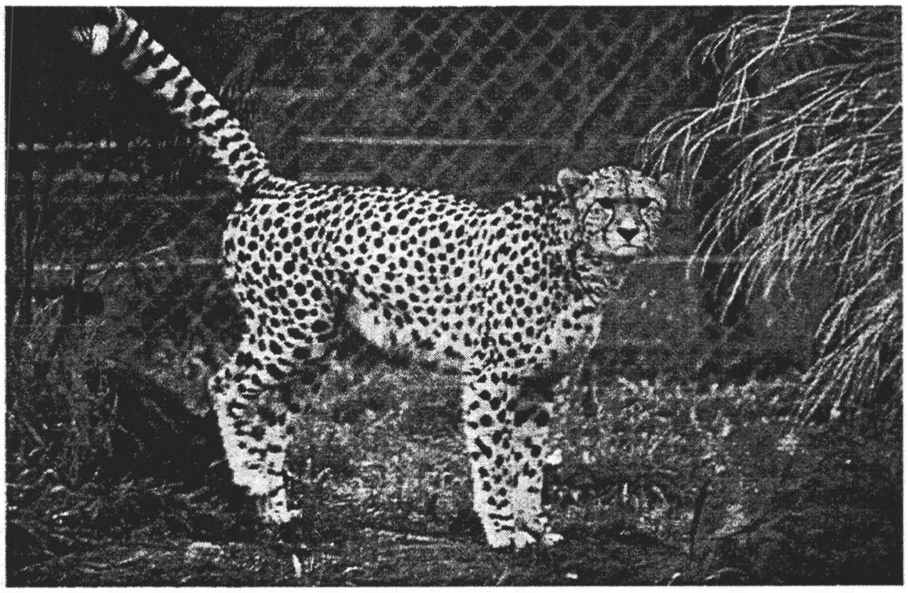
The transmission of information is essential for the maintenance of the complex social milieu of carnivore lives. Information is transmitted using visual, auditory or olfactory signalling. Conspecific olfactory signalling is a primary method of communication among all the Carnivora and is particularly relevant for the Felidae (Gorman and Trowbridge, 1989). This method of communication has several advantages over other methods. Scent marks are deposited in prominent and often very particular areas of the environment, providing a spatial and temporal record of an individual's movements and behaviour. Scent may remain in the environment for an extended period of time regardless of the presence or absence of the signal's producer (Gorman and Trowbridge, 1989).

Smell receptors in the lining of the nose are sensitive to volatile airborne substances and taste receptors on the tongue also help the cats to detect and recognize substances that dissolve in water or saliva during licking. The vomeronasal organ, a sensory organ situated at the junction of vomer and nasal bones, consists of a tubular cavity connected to the nasal cavity (Vandenbergh, 1983). The vomeronasal organ is stimulated when odorous chemicals are passed to it when the animal presses its tongue against the roof of its mouth, causing a distinctive facial expression known as the flehmen response. Flehmen is observed as a stereotyped grimace, involving the lifting of the upper lip, closing the nostrils, and deep breathing (Albone, 1984). In mammals the vomeronasal organ is involved with the detection of, and response to pheromones. However, the vomeronasal organ does not mediate all pheromonal responses in mammals, and it may be involved in other types of behaviours as well (Eisthen, 1997).

The first pheromone was discovered when the chemical produced by female silkworm moths to attract males, was identified by the German biochemist Adolph Butenandt in the early 1930's. This substance had the power to influence conspecifics from a great distance. Hence the word pheromone, derived from the Greek verb root *pherein* meaning 'to transfer' and *hormon* meaning 'to excite', became the new term to describe substances that excite from a distance (Watson, 2000).

Mammalian odours are usually more complex mixtures of chemicals than insect pheromones and Gorman and Trowbridge (1989) suggested that the term 'social odour' might be a more appropriate term for them. Social odours may result from different influences including diet, compounds from bacterial metabolism, odours produced by the animal itself and odours from other group members (Gorman and Trowbridge, 1989). Behavioural responses in mammals to different odours are influenced by past experience, developmental status, and the context in which the odour is used (Beauchamp et al. 1976).

Felids use a variety of scent marks such as cheek rubbing, scratching and urine marking. Urine marking has been observed in almost all species of Felidae, and most will spend time investigating conspecific urine marks (Wemmer and Scow, 1977). Lions (Schaller, 1972), leopards (Wemmer and Scow, 1977), servals (Geertsema, 1985), cheetahs (Caro, 1987b), tigers (Smith et al., 1989), most species of small cat (Mellen, 1993) and domestic cats (Leyhausen, 1965; Verberne and De Boer, 1976) have all been observed urine marking and investigating urine marks deposited by others.



Male cheetah showing the classic felid form of urine spraying

Urine spray marking in cats follows a characteristic form in which the cat backs up to an object, vertically lifts up its tail and directs a fine spray of urine backwards between its hind legs. In the domestic cat this is accompanied by an erratic quivering movement of the tip of the tail (Feldman, 1994) an action apparently not present in the Pantherinae (Wemmer and Scow, 1977). The odour of sprayed urine increases after it has been deposited (Hendriks et al., 1995c). This is thought to be due to the microbial activity and oxidative degradation of the two uncommon amino-acids which it contains, felinine (2-amino-7-hydroxy-5,5-dimethyl-4-thiaheptanoic acid, I) and isovalthene (2-amino-5-carboxy-6-methyl-4-thiaheptanoic acid) (Westall, 1953; Oomori and Mizuhara, 1962). After breaking down, a strong 'tom-cat' odour is produced by 3-mercapto-3-methyl-1-butanol (II) and 3-methyl-3-methylthio-1-butanol (III), and other disulphides and trisulphides (Hendriks et al., 1995b).

The pungent odour of sprayed urine encouraged speculation that it carried other secretions, possibly from the preputial or anal glands (Wolski, 1982). The anal glands are thought to contribute to the scent marks of larger felids (Gorman and Trowbridge, 1989; Wemmer and Scow, 1977). Very frightened domestic cats void anal gland secretions. These secretions have a very distinctive odour, but according to Bradshaw and Cameron-Beaumont (2000) this odour is notably different to the odour of sprayed urine, to the human nose at least.

At present there appears to be no indication that the chemical constituents or concentration of sprayed urine in the domestic cat are different from those in urine eliminated in the squat position (Wouter Hendriks per comm.). According to the diffusion model of Bossert and Wilson (1963), spraying above ground level can produce an area where the scent is detectable that is twice as large as the same scent source at ground level. Eliminatory urine is buried by the domestic cat, which will serve to further reduce any noticeable odour. This may account for the differing odour intensities between spray and elimination urine. Felinine is thought to be testosterone dependent and may be a urinary component for territorial marking (Hendriks et al., 1995a) and a precursor for a pheromone to attract female mates (Hendriks et al., 1995c). Entire males excrete larger amounts of felinine, up to 95mg/day, compared to females who produce only up to 20mg/day. The urine of females is correspondingly less smelly than male urine (Hendriks et al., 1995c).

The precise purpose of urine marking is still elusive but it is commonly considered to demarcate territory boundaries (Gorman and Trowbridge, 1989). The presence of urine marks do not deter neighbouring, or nomadic animals, but notify intruding animals of the residents presence and thereby serve to advertise borders between territories. Nomadic animals may alter their behaviour when entering new territories, e.g. nomadic lions will not roar when they are travelling through another pride's territory (Grinnell and McComb, 2001). Territorial scent marks provide an intruder with an olfactory profile of the resident. Should an intruder meet the resident it will recognise it as the resident by matching the scent from the urine marks to the resident's smell (Gosling, 1982; Smith et al., 1989).

Leyhausen (1965) suggested that urine marking might facilitate a 'time share' arrangement between solitary individuals, enabling them to share hunting grounds and overlapping territories. A fresh urine mark specifies that the area is currently being used by another individual, whilst an older mark indicates that the area is available for the next cat to use, adding its own mark to the message board as it takes possession (Leyhausen, 1979). Domestic cats can tell the difference between urine marks of various ages, sniffing longer at fresh urine than they will in response to older urine (De Boer, 1977). Similarly cheetahs will avoid following trails that have been freshly marked with the urine of a conspecific (Eaton, 1970). In this way animals may avoid unnecessary conflict.

Urine marking may record where an animal has been hunting recently, and assist the animal to avoid such areas that are unlikely to be productive at this time. Red foxes, coyotes and wolves all urine mark areas where they have been foraging or where they have cached food (Henry, 1977). Female cats urine mark more frequently when they are hunting, and most marks are left at the entrances of rabbit burrows (Kerby and Macdonald, 1988).

In the nocturnal prosimian *Microcebus coquereli*, animals can discriminate between individuals when exposed to urine samples of various familiar and unfamiliar individuals

(Schilling, 1980). It is likely that cats also share the ability to discriminate between individuals (Natoli and De Vito, 1991).

Urine marking in the domestic cat has been examined in free-ranging animals and in laboratory conditions (Verberne and De Boer, 1976; Feldman, 1993). Natoli and De Vito (1991) observed that resident cats sniffed longer at urine marks of strange cats than they did at the urine marks of familiar cats. There maybe some indication of identity incorporated in the scent message. Domestic cats can distinguish between different genders based on odour (Verberne and De Boer, 1976), and male cats will sniff longer and perform flehmen in response to the urine sample of an oestrus female. Felid females, including the domestic cat, spray more frequently during oestrus than they do at any other time (Smith et al., 1989; Bradshaw, 1992). These marks serve as advertisements of her current state of fertility and such urine marks are thoroughly investigated by passing males. The vomeronasal organ is involved in the process of detecting hormonal changes associated with the reproductive cycle (Watson, 2000).

Males intending to court an oestrus female will also increase their frequency of urine spraying (Natoli and De Vito, 1991). This is often explained as 'displacement' marking (Natoli and De Vito, 1991), however entire males will also urine mark after investiagting the urine of an oestrous female (Verberne and De Boer, 1976). It is conceivable that such marking may convey messages of information concerning the male's identity, status, health or even his genetic make up (Feldman, 1993; Groman and Towbridge, 1989; Yamazaki et al., 1998). If female urine holds relevant information for males, male urine may possibly hold information of interest to females.

Conspecific odours elicit many interesting responses in many different species, both behavioural and physiological. Merely the odour of a male can potentially alter female reproductive physiology. Exposing juvenile female mice to urine collected from adult males can accelerate the onset of puberty (Drickamer and Murphy, 1978; Vandenbergh, 1983). Adult female house mice kept in exclusively female groups can be induced into synchronous oestrus by the introduction of male odour into the female environment

(McClintock, 1983). In humans, axillary odours collected from donor males can shorten the menstrual cycle of women causing more frequent ovulation (Preti et al., 1997). Human females also prefer the body scent of males with greater bilateral symmetry. The preference is greatest during the highest fertility period within the menstrual cycle (Thornhill and Gangestad, 1999). Women in low fertility phases and women using contraceptive pills do not show this preference.

This chapter investigates the possibility of using male cat urine as a novel and interesting scent to enrich the environment of female domestic cats kept in a restricted situation. The urine of four entire males is presented to ten colony-housed females, firstly to determine whether females will show any interest in the urine of a conspecific male, and secondly to determine whether the females will respond differently to the urine of different males. The urine is presented to anoestrus female cats while they are held alone in a small room away from their pen mates. This minimizes the social impact from other cats and allows individual responses to be observed. The empty observation room should also serve to maximize the novelty of the urine and increase the likelihood of each individual cat expressing interest in the scent.

2.2 Materials and Methods

The behavioural responses of 10 entire female domestic cats (*Felis catus*) to the urine of four entire male domestic cats were investigated. The breeding group of cats at the Heinz Watties Feline Nutrition Research Unit, a research unit of Massey University, Palmerston North, New Zealand were used. The breeding group consisted of ten queens, all of whom had had prior mating experience. The females were related to two of the entire males that donated urine. Observations were carried out between May 2000 and July 2000.

2.21 Location

The Heinz Watties Feline Nutrition Unit is a Massey University research unit teaching facility. The unit maintains a colony of approximately one hundred and fifty cats. The cats are kept under quarantine conditions to ensure their health and safety. They are held in groups of up to ten cats. The unit breeds replacement cats and a small breeding colony is maintained. Most of the cats in the unit were born and bred there. New breeding cats are brought in from time to time to ensure genetic health and avoid inbreeding.

In the unit most females remain entire but only the ten females in the breeding group (pen 13) are being used for reproduction. All males are neutered if they are not required for research or breeding purposes. Most of the cats are kept in mixed groups of neutered male and entire females, except the breeding females and entire males, which are kept in single sex groups.

2.22 Animals

All four of the entire males kept at the unit (Table 2.1) were used in this trial. Ten breeding females from pen thirteen were used in this trial. They were not being used in any other study or palatability trail. Five females from the breeding group have had one or more litters of kittens (Table 2.2). All of the females have had prior mating experience with breeding males. All of the females were aged between two and eight years and are known to regularly come into oestrus.

Table 2.1. The names, birth date, prior breeding experience, and source of the males included in this study.

Name	Born	Unit Bred	Mating Experience
Sunny	09/01/93	yes	yes
Bransen	09/02/97	yes	no
Titan	14/01/98	no	no
Brock	14/01/98	no	no

Table 2.2. The names, age, source, number of litters and prior mating experience, and pen of the study females.

Name	Born	Unit Bred	Litters
Mirrim	18/01/94	yes	2
Milo	26/01/94	yes	4
Nikki	15/01/93	yes	3
Fya	14/01/96	yes	0
Kola	14/01/96	yes	1*
Tori	24/0196	yes	0
Sheba	09/01/98	no	1
Wren	16/01/98	no	0
Dusky	05/01/98	no	0
Bella	26/01/98	yes	0

* Kola carried one litter full term but the kittens were dead at birth.

2.23 Male Dominance

Staff working at the unit assessed the relative dominance of the males (Table 2.3). Ranking was based on the staff members' experience observing and working with the males. Three staff members independently assigned, and agreed on the cats' dominance ranks.

In a previous study, Kyle (1991) independently assessed the social dominance of four male cats using various techniques: competition and the order of possession of a freshly killed mouse; the direction of aggressive behaviour in response to each individual being removed and then re-introduced back into the pen; access to the food tray. Thus the dominance ranking of the four males was establish. Kyle's study (1991) revealed a dominance ranking which was the same as that estimated by the staff although her study used four different male cats to those used in the present experiment.

The weight of the males was recorded once a month (Table 2.3) for three months in order to determine whether it was consistent with their assigned dominance rank. The weight of the males follows the dominance order estimated by the staff members of the Feline Unit.

Table 2.3. The social dominance ranking of the entire males as assigned by staff members at the Feline Nutrition Unit, followed by their weight in grams as recorded over the period of the study.

Male	**Sunny**	**Brock**	**Titan**	**Bransen**
Assigned rank	1	2	3	4
Date	**Weight (g)**			
26/05/00	4468	4280	4280	4103
16/06/00	4732	4292	4202	4156
28/07/00	4750	4180	4132	4010

2.24 Relatedness

The relatedness between the male and female cats (Table 2.4) was determined using the tabular method (Nicholas, 1996). Relatedness values were double-checked using version 5.1 of Pedigree Viewer by Brian Kinghorn, with the assistance of Dr Ric Sherlock.

Precise family histories have been kept since the establishment of the unit in 1992. The tabular method is used to calculate additive relatedness, that is "the expected number of nuclear genes at a locus in one individual that are identical by descent with a randomly chosen gene at the same locus in the other individual" (Nicholas, 1996). When neither of the relatives are inbred, it also equals the proportion of genes in common between two relatives. These calculations assumed all of the founding members of this colony to be unrelated.

Table 2.4. The additive relationship between the females in this study and the unit-bred males Sunny and Bransen.

Females	**Sunny**	**Bransen**
Sheba	0	0
Dusky	0	0
Wren	0	0
Mirrim	0	0
Milo	0.25	0.375
Nikki	0.25	0.75
Kola	0.5	0.3125
Fya	0.5	0.3125
Tori	0.625	0.375
Bella	0.625	0.375

2.25 The Observation Room

The behavioural research room is approximately 2m wide x 2.5m long x 3m high (Figure 2.1). There is a door with a cat flap at one end and windows and an extractor fan at the other. The walls are painted white and the floor is vinyl. This observation room has been designed for experimental purposes. Two closed circuit cameras have been installed so that the behaviour of the cats can be observed. One camera was attached to the ceiling, entire room could be viewed from this position. The second camera was attached to the wall opposite the cat flap, in a location that gave a closer view of the cats when they approached the urine samples, allowing an accurate view of behaviours in response to the urine. A TV and video recorder was set up in the small room next to the observation room and the camera was linked into this, allowing observations to be taken from outside the room.

The camera suspended from the ceiling allowed a view of the whole of the observation room but behaviours such as flehmen could not be seen through this camera. Although camera views could be alternated on the video screen it took a couple of seconds to switch between them and behaviours could have been missed in this time. In an effort to prevent this happening, the camera view from the wall was primarily used as the females' response to the urine could be seen in detail. However, this did mean that the behaviour of the females when they were not near the urine sample could sometimes not be seen.

2.26 Habituation

It took some time for the cats to become accustomed to being handled by a new person (HR). Once they were familiar with me they were taken from their pen to the observation room. Initially the cats were frightened when they were put in the observation room, but they were played with and then gradually left alone for longer and longer periods until they would remain calm alone in the room for five minutes. It took several weeks before the cats would remain calm when they were placed alone inside the observation room.

2.27 Urine Collection

Each male cat was placed inside an individual metabolism cage overnight to collect urine. The males are trained to urinate and defecate on trays covered in a very fine wire mesh. This allows the urine to flow through to the tray underneath without being contaminated with faeces. Fresh urine was collected each Monday night, put into pottles and kept refrigerated until Friday when any that was unused was discarded. Urine was not collected more frequently because the overnight stay in the metabolism cages appears to be stressful for the cats and more frequent stays tended to result in diarrhoea and the urine sample was consequently contaminated with faeces.

Three milliliters of each male's urine was removed from the pottles using sterile syringes and held at room temperature for about twenty minutes before it was placed inside the observation room and the females were allowed to smell it. The urine was placed on double thickness sheets of 'Reflex' A4 paper (SCT Enhanced Performance, Australian Paper, 307 Mt. Ferntree Gully Rd., Mt. Waverly 3149, Australia). This paper is somewhat water resistant, and was able to stop the urine from seeping through to the vinyl underneath, thereby lessening the olfactory cross contamination between each combination of male urine.

2.28 Experimental Design

Trials were held in the morning when the cats were awake and active, during May, June and July of 2000. During these months females are usually in anoestrus although some females had previously been observed in oestrus during the winter months at the unit. The females in the study group were watched to ensure they were not showing any of the behavioural signs of oestrus.

The urine from two males was placed on separate sheets of paper, which were then placed in the middle of the observation room. The urine of each male was randomly assigned from the six possible combinations that ensured each male had been paired with each of the other males. Each female was given access to each of the six possible combinations in random order, five times, and visiting the observation room thirty times in all. Females were taken to the observation room in random order, to minimize the effect of the previous females' behaviours on those that followed.

Urine was placed in two defined sites, although the placement of the urine was randomized. This was done so that the visual cues upon entering the room were consistent, so that each female would be responding only to the olfactory cues.

One combination of the two urine samples was used each day, at the end of the day the floor was washed down with water and sprayed with 'Pet S.O.X. Animaux' (Rolf C. Hagen Inc., Montreal, Quebec H4R 1E8) to remove all animal odours. The area was washed down with water again the next morning before beginning the next day's trials.

When the urine had been placed in the room, one randomly selected female was put through the cat-flap into the observation room and observations began. A stopwatch was used to record the timing of each cat's behaviour. The female's behaviour was recorded for five minutes and then she was returned to the pen. Behaviour was recorded on an observation sheet and videotaped at the same time to ensure that no behaviours were missed.

Specific aspects of the females' behaviour was recorded (Table 2.5) and other behaviours seen during the five minutes of observation were also recorded

2.29 Analysis

Recorded aspects of the females' behaviour were analysed by analysis of co-variance (ANCOVA) using Systat 8.0 for Windows.

The latency in seconds from the time each female entered the observation room until the time she approached the urine sample of each male was recorded. If a urine sample was not approached within 3 minutes, non-approach was recorded and that trial excluded from the analysis of latency.

The female response to the urine samples of all four males was analysed using a model that included factors for individual male and female, and relatedness as a covariate. Females were included in the model to account for any variance in individual responsiveness as a reflection of individual curiosity levels. Interactions could not be fitted as each female only has one degree of relatedness with each male.

The data was not normally distributed, the residuals for the sniff duration and latency data were positively skewed and the variance of the residuals increased with increasing predicted values. A square root transformation was performed on the sniff duration data and the latency data worked best with a log transformation.

Results from this analysis lead to the question 'do females innately show a different level of curiosity regardless of what or who they are investigating?' If they do, this could influence the overall picture of female preference, as a preferred male may appear less appealing if the female is just generally not very curious or vice versa.

When the effect of relatedness on the female response was analysed, the males Titan and Brock did not need to be directly considered in the analysis, as they are not related to any of the females. Therefore they could be used as controls for variations in the behavioural responses within individual females.

For each parameter recorded each female's responses to the two non-related males were used as controls (covariate) for within-individual responses to the two males to whom they are related. Thus a 'measure' of curiosity was generated as a reflection of each female's behaviour in terms of sniff duration, latency, visits and flehmen. The second model used males as factors, and relatedness and curiosity as covariates.

Table 2.5. Ethogram of recorded female cat behaviour.

Latency (seconds)

The latency was the time from when the cat went through the cat-door until the time that her nose was within 10cm of a urine sample.

Visits

Number of times a cat approached a urine sample, sniffed and then moved further than 10cm away from the sample.

The Duration of Sniffing (seconds)

The time during which the cat has its head extended towards, and within 10cm of the urine.

Flehmen

Number of times a cat performed flehmen over a urine sample.

Ear Position as an indication of interest, aggression or submission (Bradshaw, 1992)

Ears forward - standard
Ears sideways and down - submission
Ears backwards - aggression

Tail Position as an indication of interest, aggression or submission (Bradshaw, 1992)

Tail vertical – social
Tail horizontal – amicable approach/ sexual approach
Tail concave – defensive aggression
Tail lowered – aggression
Tail between legs- submission

2.3 Results

The queens generally responded to being placed in the observation room by initially investigating the room, or sitting by the door crying for a while before beginning to investigate the room. Frequently the urine samples appeared to be investigated as a continuous part of investigating the room in general. On other occasions the urine samples appeared to be deliberately approached and sniffed.

Some cats would continue investigating the room until the end of the observation period, others would just wander around crying or sit near the door crying continuously. By the end of any observation period the female was usually sitting near the door and crying repeatedly.

There were variations in each female's response to being placed in the observation room, and their response to the urine samples. Some, such as Nikki tended to approach the urine samples earlier and sniff longer (Figures 2.1 and 2.2). Others, such as Kola tended to investigate less, approach the urine samples less, sniff less, and frequently spent much of the observation time sitting near the door.

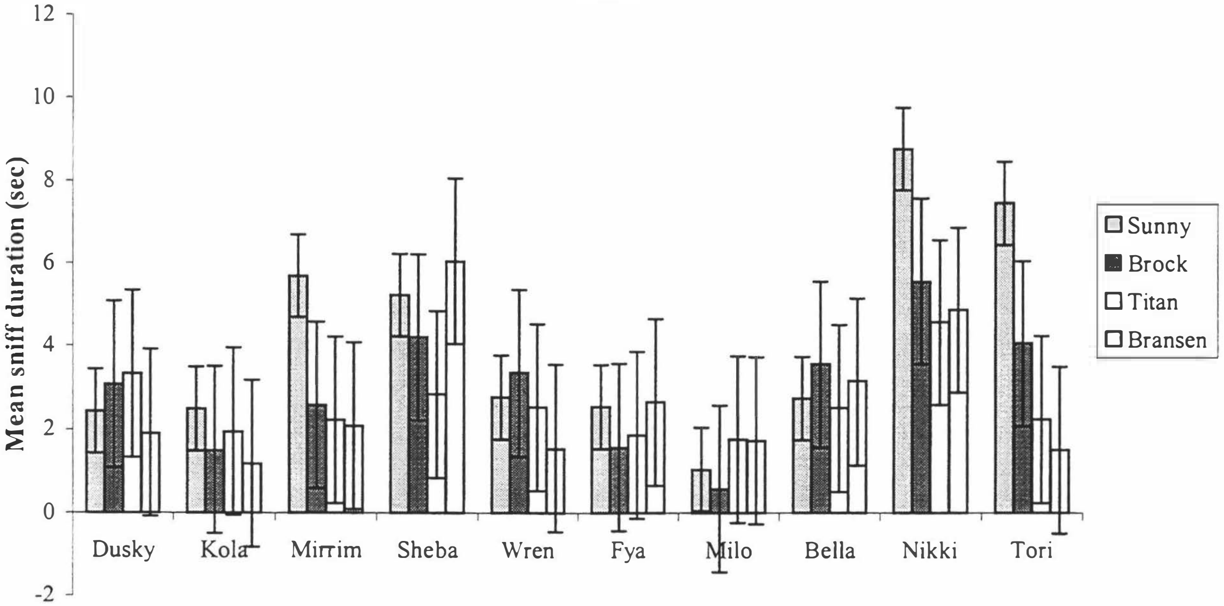
2.31 Body Language

All of the females adopted similar body postures when they were investigating a urine sample. Tails were consistently held slightly downwards and straight. Ears were held slightly to the side. The rest of the body appeared to be relaxed as they approached the urine and sniffed.

2.32 Female Response to the Dominance Rank of the Males

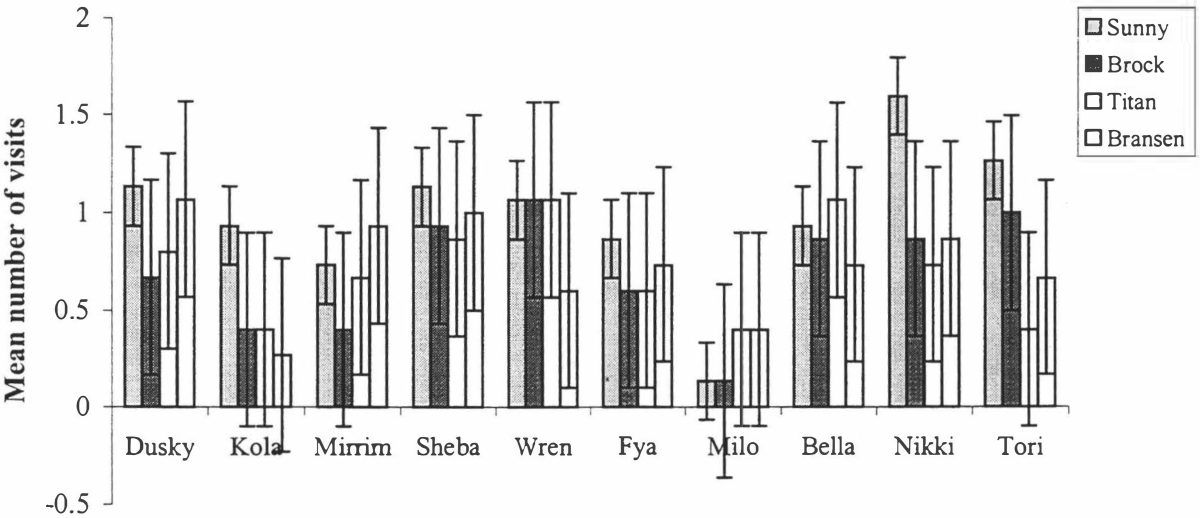
There were no significant differences in the females' responses towards the urine of the four males for any of the parameters (Table 2.6). Relatedness appeared to have no impact on the female response to the males' urine for any of the parameters recorded (Table 2.6). There were, however, significant differences between females in their overall level of responsiveness, for all parameters except latency (Table 2.6, Figures 2.1 and 2.2).

Figure 2.1. Mean sniff duration of each female in response to the urine of each male



Females

Figure 2.2. Mean number of visits by each female to the urine of each male



Female

Table 2.6. The *F* -ratio and *p* -value for the factors (males and females) and the covariate (relatedness) for each of the parameters of female behaviour that were recorded in response to the urine of all four males.

Parameter Measured	**Males** *F* **3,26**	***P***	**Females** *F* **9,26**	***P***	**Relatedness** *F* **1,26**	***P***
Sniff Duration	2.42	0.090	5.78	<0.001	0.15	0.702
Latency	0.75	0.530	1.00	0.466	0.00	0.970
Visits	2.88	0.054	4.70	0.001	0.00	0.952
Flehmen	1.05	0.384	5.09	0.001	1.69	0.205

Table 2.7. The *F* -ratio and *p* -value for the factors (males) and the covariates (relatedness and curiosity) for each of the parameters of female behaviour that were recorded in response to the urine of the two males that share varying degrees of relatedness with the females.

Parameter Measured	**Relatedness** *F* **1,15**	***P***	**Males** *F* **1,15**	***P***	**Curiosity** *F* **1,15**	***P***	**Interaction (Males*Relatedness)** *F* **1,15**	***P***
Sniff Duration	0.53	0.477	1.75	0.205	12.64	0.003	0.01	0.908
Latency	0.06	0.813	0.78	0.391	1.76	0.204	0.85	0.774
Visits	0.08	0.931	0.78	0.392	6.80	0.02	0.38	0.548
Flehmen	2.31	0.149	0.17	0.688	5.43	0.034	0.00	0.988

Although there are no significant differences in the females' responses towards the urine of different males, there is still an interesting trend when the overall responses are considered together. Although the three subordinate males, Brock, Titan and Bransen appear to have no consistent ranking for any of the parameters, Sunny consistently received the most attention from the females over all of the parameters. Sunny received the longest mean sniff duration (Figure 2.3), the most visits (Figure 2.4), the shortest latency (Figure 2.5) and the most flehmen responses (Figure 2.6), however the difference was not significant for any of these parameters (Table 2.6). No male was investigated longer than a mean of 4.5 seconds out of the 300 seconds (5 minutes) for which the females had access to the urine samples (Figure 2.3) and the urine samples averaged less than one visit per 5-minute observation period (Figure 2.4).

2.23 Effect of Relatedness on the Female Response

The female response towards the urine of the two males with which they shared varying degrees of relationship showed no effect of relatedness for any of the parameters recorded (Table 2.7).

Sunny's urine appeared to elicit more interest as indicated by the females' sniff duration and number of visits (Figure 2.7 and 2.8). The latency to approach a urine sample did not follow this trend (Figure 2.9). Sunny received slightly more flehmen responses from females that have a low level of relatedness to him, but females appear to express flehmen more often in response to Bransen when they are more related to him (Figure 2.10). The trend line for Bransen is brought up by the presence of Nikki who is both highly related to Bransen and a very curious female. However, there were no significant differences between the responses by the females towards the urine of Sunny and Bransen for any of the parameters (Table 2.7).

Figure 2.3. Mean female sniff duration in response to the urine of each male

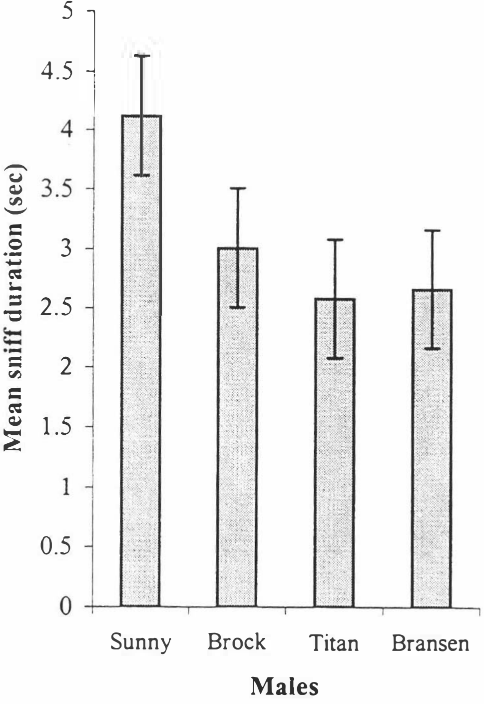


Figure 2.4. Mean number of female visits to the urine of each male

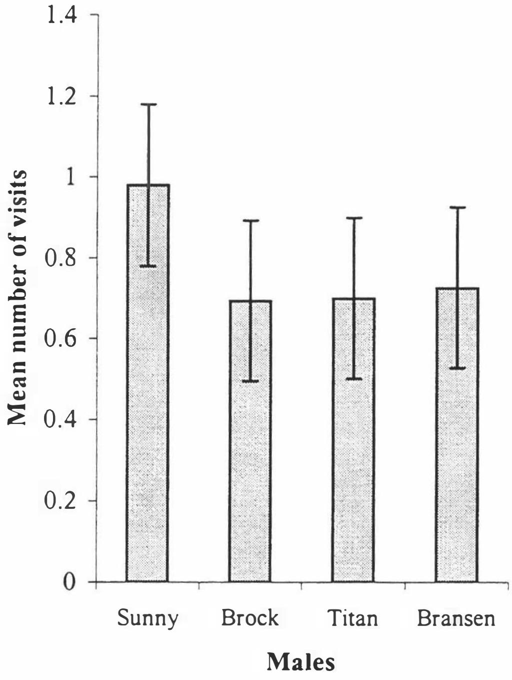


Figure 2.5. Mean female latency to approach the urine of each male

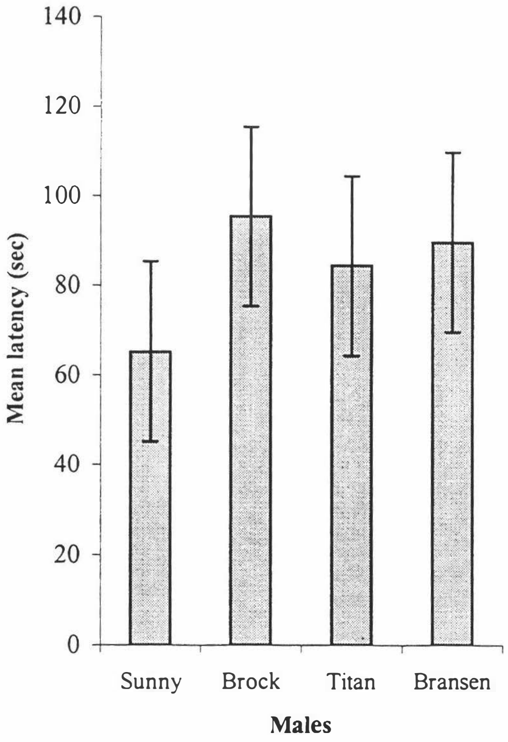


Figure 2.6. Mean number of flehmen by females in response to the urine of each male

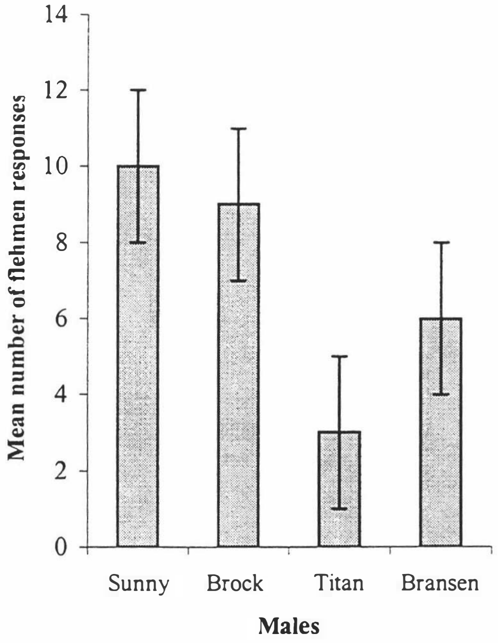


Figure 2.7. Mean sniff duration at the urine samples of the males Sunny and Bransen plotted against each female's relatedness to each male.

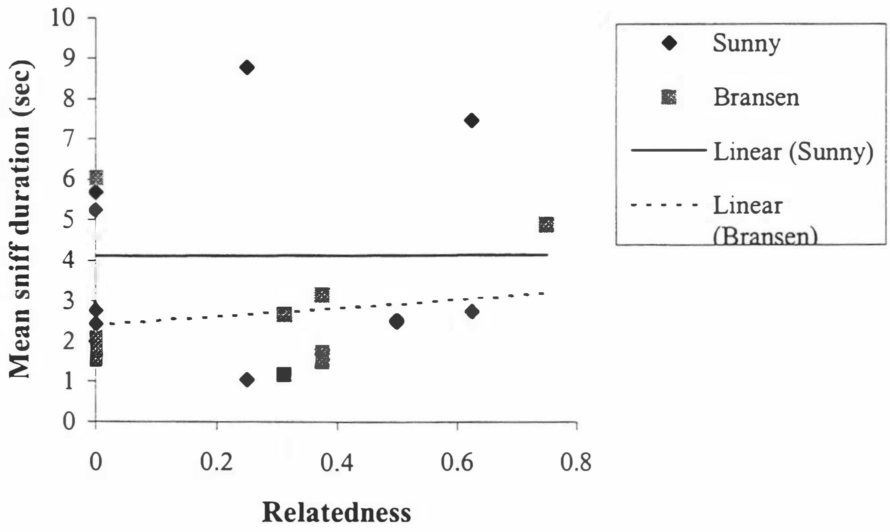


Figure 2.8. Mean latency to approach the urine of Sunny and Bransen plotted against the relatedness of the females to each male.

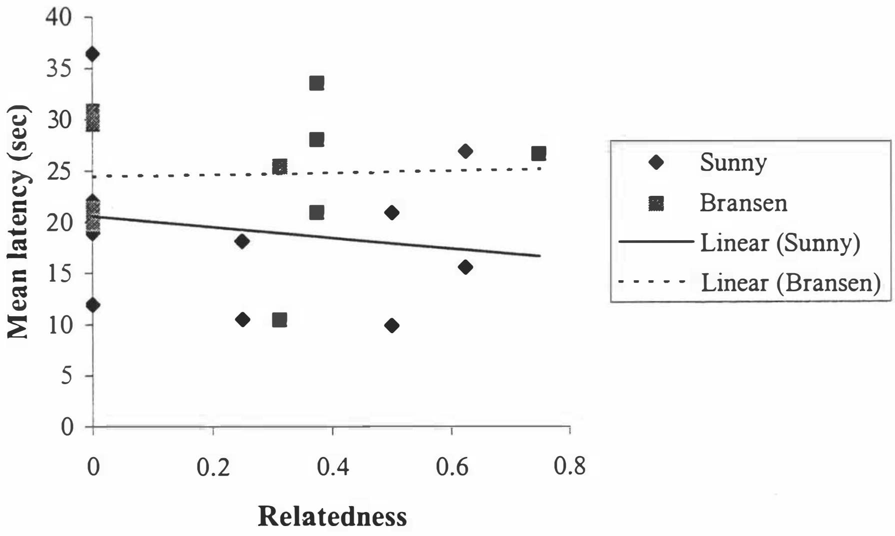


Figure 2.9. Mean number of female visits to the urine of Sunny and Bransen plotted against the relatedness of the females to each of the males

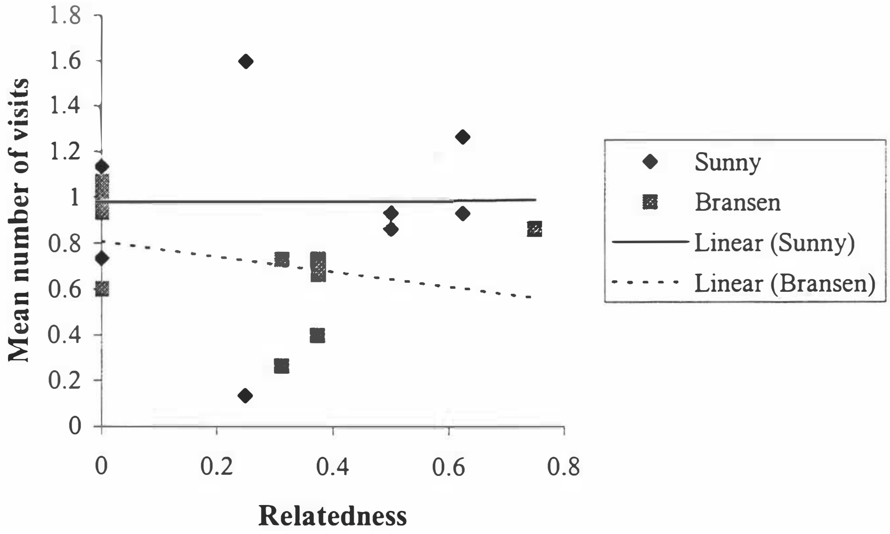
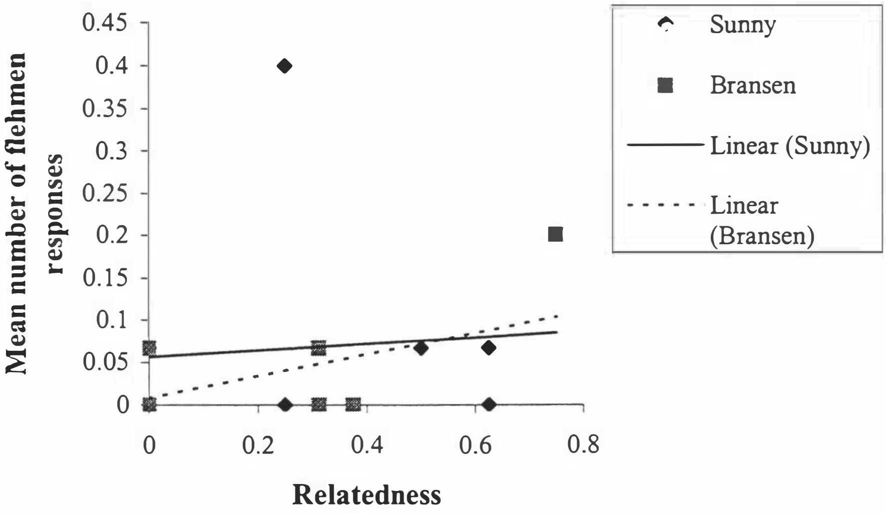


Figure 2.10. Mean number of flehmen responses to the urine of the males Sunny and Bransen plotted against the relatedness of the females to each male.



2.34 Female Curiosity Levels

For all behaviours, except latency, the covariate (curiosity) was significant (Table 2.6 and 2.7) showing that females had different individual levels of curiosity as estimated from their behaviour towards the unrelated males.

Each female responded to the urine samples in their own individual way. From Figure 2.1, it can be clearly seen that there are substantial differences in the duration for which each female sniffed the urine of the males. For example the female Nikki sniffed all males considerably longer than the female Kola. In fact, the male that appeared to hold the least interest for Nikki (Titan, mean sniff duration = 4.6 s) was still investigated for a longer period of time, than the male that Kola found the most interesting (Sunny, mean sniff duration =2.5 s) (Appendix 1). Although both of these females appeared to prefer the scent of Sunny to the other males, the degree of interest they showed was very different.

2.4 Discussion

Ten entire anoestrous female domestic cats (*Felis catus*) were presented with the urine samples collected from four entire male cats and their behavior in response to the urine sample was observed. Four parameters were recorded as indications of interest from the females towards the urine samples. These were: latency to approach the urine sample, sniff duration, the number of flehmen responses performed by the females over the urine samples, and the number of times that each female returned to investigate any one sample.

- No significant difference was observed in the behaviour towards any of the urine samples of the four males in any of the parameters measured.
- Conspecific urine may hold limited use as an environmental enrichment technique as it did not elicit a very large response from the anoestrus females in terms of either sniff duration or number of visits.
- Dominance rank assigned by staff at the unit and confirmed by male weight over the duration of the study had no affect on the females’ responses.

- Two of the males had varying degrees of relatedness with the females. Relatedness had no effect on any of the female responses.
- The body language of the females was consistent throughout the observations. All approached the urine patches with their tails half-lowered and straight. Ears were held slightly to the side and bodies appeared relaxed.
- The behaviour of individual females varied significantly. Within-individual behaviour was consistent for sniff duration, visits and flehmen response, but not for latency.

2.41 Individuality

Although there was considerable variation between the behaviour of individual females, each individual was predictably consistent in her responses towards the urine samples. Some individuals were always interested and would thoroughly investigate each urine sample; others showed very little interest, and even when these females investigated both of the urine samples it was only for a second. Sniff duration, number of visits and observed flehmen responses were all found to be consistent within individuals. This may be a reflection of innate differences in the levels of curiosity between the females.

However latency was not consistent within individuals. This may represent an inconsistency in female behaviour or it may be that latency is not a good measure of interest. Females may not be able to determine how interesting a sample is before it has been sniffed and latency to approach the sample may be more an indication of a female's response to being in alone in an unfamiliar room.

Many different species have been shown to have distinct individual differences in their styles of behaviour (Stevenson-Hinde and Zunz, 1978; Feaver et al., 1986; Fagen and Fagen, 1996). Different patterns of behaviour may only be revealed over time when ongoing consistencies characteristic of an individual are compared to the behaviour of others in similar situations (Feaver et al., 1986). Such behavioural styles may be temporary responses to the immediate situation and changes in the environment, season, age or hormonal status may influence these responses, or they may be consistent responses reflective of an individual's temperament or personality (Mendl and Harcourt, 2000).

All of the females in this study were assumed to be anoestrus and they were constantly monitored for the behavioural signs of oestrus. Occasionally female behaviour does not accurately reflect her hormonal status and it may be that some of the different levels of interest shown were motivated by hormonal changes that had not been identified by the observer. However, the study was conducted over three winter months and even if some changes in hormonal status were missed it would be unlikely that such variations would be consistent for the duration of the study. Furthermore, significantly consistent levels of curiosity were found within the individuals of this study. Changes within an individual's level of responsiveness that were inspired by hormonal changes would have contradicted such a finding.

2.42 Dominance rank

Male social dominance in the domestic cats is generally thought to be a linear dominance system (Leyhausen, 1979; Natoli and De Vito, 1991). Yamane et al. (1996) and Liberg (1983) found that social dominance rank in males based on the outcome of agonistic interactions correlated to male weight. Heavier males are more likely to be the victor in agonistic interactions (Ishida et al., 2001). Staff members who interact with these animals on a regular basis assessed the dominance rank of the entire males using the observed outcomes of agonistic interactions and the number of threats given and received between the four males. The rankings assigned to the males were consistent with the males' weights over three months. Sunny was assessed as being the most dominant male and he was also the heaviest male. Brock and Titan were assessed as holding intermediate rankings, with Brock being more dominant and weighing slightly more than Titan did. Bransen was clearly the most subordinate male out of the four and he weighs the least.

The anoestrous females responded to the urine of all four males in a similar manner and no significant differences were observed. Dominance rank does not appear to have any impact on the interest generated by the male urine samples. Although the differences in female response were not significant, there is a trend for Sunny to be preferred over the other males. Sunny's urine received the greatest mean sniff duration, the greatest mean number of visits and flehmen responses and the shortest latency. None of the dominance ranks are replicated and each ranking will be indistinguishable from any individual effects of each male.

Sunny is the most dominant male but it may be some other aspect unique to him to which the females are responding. Sunny is also the oldest male. The two males that hold intermediate ranks Titan and Brock are five years younger. This may affect how interesting the females find these two males. All the males are housed near to the breeding females pen, therefore the scents of all the males are likely to be familiar to the females. However, Sunny is also the only male that has had mating experience with these females. The familiarity of the females with Sunny in this context may have caused them to pay slightly more attention to his urine.

2.43 Relatedness

Some of the females in this study shared varying degrees of relatedness with the two males, Sunny and Bransen, that were born and bred at the unit. The additive relatedness between the males and female ranged from '0', being unrelated, through to '0.75', being highly related. Relatedness did not appear to have any effect on the responses of the females towards the male urine samples. The two unit bred males were considered separately from the two males that were introduced to the colony. Both of the males bred at the unit shared varying degrees of relatedness with the females, and they held both the highest and the lowest dominance ranks amongst the four males. Females showed a slight trend to express flehmen more often when they either shared a low level of relatedness with Sunny, or alternatively a very high level of relatedness with Bransen. However neither of these differences in response were statistically significant.

2.45 Visual Signals

Cats have very expressive body language, which is used to moderate outcomes in a wide range of social interactions. Although the use of body language in agonistic encounters has been thoroughly documented, as has that of females in oestrus (Leyhausen, 1979), non-agonistic encounters have been less well documented. Cats use their ears and tails to indicate intentions of aggression or submission. Ear positions can be altered very quickly and may show different indications of a cat's intent to what is displayed with its body language. A backward rotations of the ears is used in an offensive posture, whereas ear that are folded sideways and downwards send a message of submission, with the intensity of the angle on a continuum between these two extremes showing the cat's intent (Bradshaw, 1992).

Although the tail is primarily an accessory used to balance cats in locomotion, it too is available to provide signals. The normal tail position is straight and horizontal or half-lowered. Cats will raise their tails vertically as a greeting signal. When the tail is pointed down towards the ground it is usually and aggressive signal and the tail is tucked in between the hind legs is a signal of submission (Leyhausen, 1979).

Little can be inferred from the body language observed in the females as they approached and investigated the male urine samples, which was consistent with the standard body language of cats in normal situations. The observation room is small (3m x 2.5 m), and was empty apart from the urine samples. Although the urine samples may have indicated that males had been present at some time, each female would have been able to clearly see that the room was empty upon entering it.

Body language sends an immediate signal that has no permanence, and the signals are intended to be received by another cat that is present at the same time. The fact that the females could see that they were alone, and had visited the room safely on several other occasions may have contributed to their relaxed body language.

2.46 Experimental Design

Anoestrous females are known to be generally less interested in the urine marks of male cats than when they are in oestrus (Bradshaw, 1992). This general phenomenon may account for the similar level of interest shown towards all of the donor males' urine and female interest and preferences may be different when females are in oestrus. However all the urine samples were regularly approached and investigated by all the females. This indicates that, although the females did not appear to distinguish between individuals, the urine samples did elicit some interest from the anoestrous females.

The method of presenting only two urine samples at a time was rather ponderous; females had to visit the observation room six times before the responses to the urine of all four males could be compared. Although the cats at the feline unit are handled regularly, they are generally kept in groups and became distressed when they were kept alone in the observation room for an extended period of time. Each female only visited the observation room twice in one day, as more frequent periods of isolation that were required during the observations, appeared to distress the cats.

The urine used in this study was collected once a week. De Boer (1977) showed that domestic cats respond less to older urine samples than they do in response to fresh urine. The urine in this study was refrigerated in order to reduce its rate of decomposition. 3mls of male urine was removed from the fridge and allowed to warm up before it was presented to the females, however the temperature of the urine samples is likely to have increased over the morning which would have affected its olfactory qualities. The age and the temperature of the urine were variable; that may have affected the female response and could have been taken into account when recording the females' behaviour.

The urine used in this study was not sprayed urine, as it is very difficult to collect. Eliminatory urine was used as it is easy to collect and there appears to be no published accounts of any chemical differences between eliminatory urine and sprayed urine. Sprayed urine is dispersed at a height and in a way that is likely to maximize its olfactory impact (Wemmer and Scow, 1977). Being smellier, it may convey more information than eliminatory urine, therefore sprayed urine may have elicited a greater response. The males were all familiar to the females. Natoli and de Vito (1991) observed that cats would sniff longer at the urine of strange males than they will at the urine of familiar males. Urine of unfamiliar males may have elicited more interest from the females.

Furthermore, the relative interest that the females showed in the male urine would have been clearer had a control also been presented. The female response to a control sample could then be compared with the female response to the urine samples. In this way the effect of the odour could be distinguished from other aspects of the presentation, such as the novelty of the white paper on which the urine was presented. The room was usually thoroughly investigated by each female at some time during the five minute observation period, and any novel feature may have attracted the cats' attention. Without a control, the relative interest elicited by olfactory stimulation using conspecific urine, and any potential such a treatment may have as an environmental enrichment technique, remains unclear. Recording the amount of 'crying' by the females while they were in the observation room with, and without, the urine samples may have yielded a more accurate picture of any enriching effects the olfactory stimulus may have for female cats.

2.47 Environmental Enrichment and Conclusions

The urine samples were presented to the females in a barren room, away from their pen-mates. This method was thought to reduce the social effects and emphasize individual responses that may indicate the environmental enrichment potential of conspecific urine.

Anoestrous female domestic cats showed interest in the urine of four conspecific males as indicated by the females' consistent approaches and their investigation of each urine sample by sniffing and flehmen. All four males elicited a similar response from the ten females, regardless of each male's social rank, or his degree of genetic relatedness with each female. Responses were minimal overall with females averaging less than four seconds of sniffing at the urine samples and visiting an average of less than once per five minute observation period. These results suggest that the urine of familiar males may have little value as an environmental enrichment tool for conspecific anoestrus females.

Chapter Three

The Response of Oestrous Female Domestic (*Felis catus*) cats to Conspecific Male Urine

Bella being flirty

"One cat just leads to another"

Ernest Hemingway

Abstract

The environmental enrichment potential of conspecific male urine for oestrous female felines was investigated at the Heinz Watties Feline Nutrition Research Unit, a research unit of Massey University, Palmerston North, New Zealand. Eighteen oestrous domestic cats were presented with urine samples collected from four entire male cats. Sniff duration, latency to approach a urine sample, number of flehmen responses and the number of visits to each sample was recorded as indicators of female interest. Females were observed to investigate less, in response to the urine samples of males to whom they are closely related. A significant difference was found between the female responses to the most dominant male and the most subordinate male. Urine from the dominant male received the most attention overall, although this male was also the oldest and the most experienced breeder, therefore it may have been some other aspect associated with this male that attracted the females. Varying responses between individuals suggest that conspecific urine may be of interest to oestrous females.

3.1 Introduction

3.11 Environmental Enrichment

Many of the most successful approaches to environmental enrichment for captive felids have focused on appetitive behaviours. The presentation of whole carcasses (Bond and Lindburg, 1990; McPhee. 2002); hiding food within the enclosure (Carlstead et al., 1993a); mechanical or computerized 'hunts' with food rewards (Mellen et al., 1981; Markowitz et al., 1995) and the provision of live prey such as fish (Shepherdson et al., 1993) have been demonstrated to enhance psychological and physical wellbeing in felids. Obtaining food is a fundamental biological drive and the motivation to perform appetitive behaviour remains high even when it is disconnected from its functional consequence, as it is in most captive situations (Hughes and Duncan, 1988).

Another fundamental biological drive is reproduction, although this area of animals' lives as a focus for environmental enrichment has been somewhat neglected. Zoos face many financial and practical challenges that must be taken into consideration when starting captive-breeding programs for endangered species. Genetically appropriate mates must be located, appropriate housing facilities for the mother and offspring and enclosure space for the adult offspring need to be provided, all of which have financial costs associated with them. Furthermore there are always potential complications which can arise at any stage from mating to the rearing of offspring.

Reproduction in mammals begins with mate location leading onto the stages of courtship, mating, pregnancy, parturition and rearing of offspring (Lindburg and Fitch-Snyder, 1994), all engender a wide range of behaviours and provide functional outcomes. An increased range of behaviours and functional outcomes are considered to be primary goals of environmental enrichment and representative of improved welfare (Chamove, 1989; Carlstead and Shepherdson, 1994). For those animals that are not involved in a captive-breeding program the motivation to find a mate and reproduce is still a fundamental drive. Although mating and reproduction may not be viable options for many zoos, mate location and mate choice may provide a range of enrichment opportunities.

The instinctual search for mates can lead to aggravation and stereotypical pacing in captive carnivores (Carlstead and Seidensticker, 1991). Hunting lures made from bear urine placed inside the enclosure of an American black bear (*Ursus americanus*) significantly reduced stereotypic pacing during the mate-seeking season. An increase in foraging and exploratory behaviours was also observed (Carlstead and Seidensticker, 1991). In reproductively successful pairs of captive snow leopards, males will show a dramatic drop in pacing during the oestrus season and an increase in scent marking behaviours (Freeman, 1983).

It is clear from studies of carnivores that facilitating mate location is an important function of scent marking (Ewer, 1973; Macdonald, 1980; Sandell, 1989). For solitary species scent marks allow familiarity that may be particularly important where there are territorial overlaps that may mean that individuals may have occasional contact, but can also leave signals in locations that allow them to monitor each others activities (Lindburg and Fitch-Snyder, 1994).

3.12 Mate choice and Male Dominance

In the domestic cat (*Felis catus*) females may actively choose which males will father their offspring (Natoli and De Vito, 1991; Yamane, 1998; Ishida et al., 2001). However, female mate choice is a controversial topic (Birkhead and Moller, 1993; Gowaty, 1994; Ishida et al., 2001) and until recently most researchers assumed that female domestic cats did not actively choose their mates. It was thought that a female would mate with any male that could gain access to her allowing the male-male competition to ensure that only the strongest males would be able to fend off all the other males present (Liberg, 1983; Liberg and Sandell, 1988). In this manner females were thought to passively choose the 'best genes' by encouraging male-male competition (Natoli and De Vito, 1991).

There is a general increase in agonistic interactions between males during the oestrous season (Yamane et al. 1996). Females are thought to provoke competition between males by increasing scent marking during oestrus (Liberg et al., 2000). However several studies found that fighting is rare between males courting an oestrus female and males that engage in fighting during courtship may miss out on mating opportunities (Dards, 1983; Liberg, 1983; Natoli and De Vito, 1991). Most agonistic encounters between males consist of threats given or received and are resolved by one or other of the cats retreating from the situation (Yamane et al., 1996).

Conventional thinking suggests that the most dominant males should be the most attractive mates (Liberg, 1983; Liberg and Sandell, 1988). Say et al. (2001) found in their study of group-living cats in Lyon, France, that dominant males had greater reproductive success than subordinate males.

In the oestrous season male cats frequently employ two different mating tactics. Some males predominately court females exclusively from their own group. Other males may temporarily extend their range and court females from other groups (Dards, 1983; Liberg and Sandell 1988; Yamane et al., 1994). Lighter (subordinate) males tended to court females from their own group exclusively. Heavier (dominant) males visited females in other groups, as well as courting the oestrous females from their own group (Yamane et al., 1994).

Stoats also appear to employ these two basic mating tactics. Sandell (1986) found that dominant male stoats exhibited a ‘roaming’ movement pattern, which enabled the males to contact more females during the oestrus season. Subordinate male stoats were ‘stationary’ and consequently contacted fewer females.

The body weight of individual males was found to correlate with the number of observed copulations and the copulation rate per hour (Yamane, 1998). There were significant differences in the number of females courted between the three different weight classes, with ‘heavy’ males courting more females than ‘middle’ males, who courted more females than ‘light’ class males (Yamane, 1998), suggesting that dominance is a successful male reproductive technique.

A dominant male in its resident group may become a subordinate male when visiting another group. This lowered status appears to result in fewer copulations between groups (Yamane, 1998). Even if a male’s success rate was not very high, a dominant male should still pursue extra group mating when its own group females are not in oestrus (Yamane 1998).

Liberg (1983) found in a population of Swedish farm cats that the closer spatial positions held by dominant males around the oestrous female was positively correlated with a greater number of copulations. Dominance is also correlated with age and body weight (Liberg, 1983). Yamane et al. (1996) established dominance by the body weight of males as it was found to be one of the most important components determining fighting ability, courtship rank, and the copulatory success of male cats.

In the absence of active female choice, traditional theories predict that females would mate indiscriminately with any male that courts her, relying on male-male competition to ensure the ‘best’ males gain priority to the oestrous female (Natoli et al., 2000). However confounding evidence regarding the importance of dominance in male reproductive success have called these theories into question (Natoli and De Vito, 1991, Natoli et al., 2000; and Ishida et al., 2001).

Natoli and De Vito (1991) found that a dominance rank based on the outcome of agonistic interactions between males correlated with the position each cat held around an oestrous female. Dominant males held more central positions (within 0-1 meters of the oestrous female) and subordinate males held more peripheral positions (1-5 meters from the female). However, contrary to Liberg's (1983) results, neither dominance rank, nor proximity to the female were found to correlate with copulatory success. Even if a male succeeds in mounting the female, he may not achieve intromission (Ishida et al., 2001), and the number of mounts a male obtains does not appear to correlate with the number of copulations (Natoli and De Vito, 1991; Ishida et al., 2001). The inconsistencies between mounting and intromission may possibly account for the apparent conflict with Liberg's (1983) study where successful copulations do not appear to be distinguished from successful mounts.

Females can reject males at two stages prior to copulation. Males can be rejected before mounting by pawing, hissing and rushing, where the female suddenly breaks away from the courting male, causing confusion among the attending males and forcing dominant males to re-establish their position (Liberg, 1983; Yamane, 1998; Liberg et al., 2000). Once mounted, a female can prevent intromission by not cooperating with the mounting male. When mounting, "a male grasps the female firmly at the nape of the neck, and he can achieve intromission only if she raises her hindquarters to present her genitals and poses to accept copulation" (Ishida et al., 2001). Females have been found to be more choosy at the time of copulation than at mounting (Ishida et al., 2001), and observed copulations do not necessarily correlate with paternity (Yamane, 1998). The findings of Yamane (1998) imply that there may be some method of post-copulatory sperm selection in female *Felis catus*. Natoli and De Vito (1991) suggest that multiple matings by a female cat may increase sperm competition in the reproductive tract resulting in the healthiest and fastest, i.e. 'best,' sperm being selected.

Female cats living in multi-male, multi-female groups frequently mate with multiple males (Natoli and De Vito, 1988; 1991; Yamane, 1998; Natoli et al., 2000). Yet it was expected that if females preferred certain males, then they would attempt to avoid mating with other males. Natoli et al. (2000) suggest that if female choice does exist, then all females should select some consistently discernable male characteristic, such as dominance. Natoli et al. (2000) concede that individual females did mate more frequently with some males and rejected other males more frequently. However the preference for a particular male or males was different for each female and the preferred males did not share any consistent characteristics such as age, dominance rank, or any morphological characteristics such as size.

Natoli and De Vito (1991) and Natoli et al. (2000) found no indication that dominant males obtained more copulation than subordinate males. However the 'occasional' males visiting from other groups were ranked together with the resident males at the study site. The dominance rank these cats held in their own groups was unknown. If the findings of Yamane (1998) are consistent for all groups of cats, the copulation success rate may not be an indication of actual reproductive success. Indeed if the temporary courtship rank of visiting males does not impact on their reproductive success, it would be more advantageous to avoid conflict during their short sojourn and focus their time on obtaining as many matings as they can. It has already been established that the more dominant males tend to visit other groups as well as their own during the oestrus season. So it is relatively certain that 'visiting' (Yamane, 1998) or 'occasional' (Natoli and De Vito, 1991; Natoli et al., 2000) males are not of a subordinate status, and are of an age and status that theory dictates should be preferred by females.

3.13 Relatedness

Another parameter that female domestic cats may select for is the degree of relatedness. Polymorphic microsatellite DNA analysis of kitten paternity in group-living females, found that more than half the kittens were fathered by extra-group males, who were presumably non-kin. This result was unexpected as extra-group males appeared to receive fewer copulations than group-living males (Yamane, 1998). Ishida et al. (2001) found that females were less likely to accept mounting attempts and copulation attempts by related males than by non-related males. Liberg (1983) observed that oestrous females living in groups with related males, left their territories more often than oestrous females that did not live with any related males. All of these finding suggest that although female choice may not be obvious, some selection does occur.

Oestrous females tend to mate with males from their own group more frequently than they do with extra-group males. In Yamane (1998) between 68% and 83% of copulations for females were with males from their own group. However, extra-group males fathered 68.2% of the kittens born over the same period of time. Many of the visiting males have lower courtship ranks but, despite their comparatively poor copulation rate, sire a proportionally higher percentage of offspring compared to the resident males who hold higher courtship ranks. This preference for visiting males may indicate inbreeding avoidance.

Until recently it was questionable whether inbreeding depression was damaging enough in any species to warrant inbreeding avoidance behaviours (Pusey and Wolf, 1996). Meagher et al. (2000) compared the survival and reproductive success of inbred and outbred wild-derived house mice living in competitive enclosures. One generation of inbreeding (full-sibling matings) in populations derived from wild mice was found to have a 57% decrease in fitness. This decrease was significantly greater than the loss of fitness found due to inbreeding in the control laboratory mice (Meagher et al., 2000). Inbreeding avoidance functions to increase genome wide heterozygosity thereby not only bestowing increased resistance to infectious diseases but also protecting the heterozygote from recessive, deleterious mutations (Potts et al., 1994; O'Brien, 2000).

In some species, females that are allowed to choose their own mates produce offspring with a greater viability. Female house mice (*Mus musculus domesticus*) were found to produce more litters when mated with preferred males. The sons from matings with preferred males were also found to be socially dominant to the sons of non-preferred males. The offspring of preferred males were better nest builders and had a significantly higher survival rate in field enclosures than the offspring of non-preferred males (Drickamer et al., 2000).

It is only recent research that has clearly elucidated the contribution that the major histocompatibility complex (MHC) makes to mate choice and kin recognition. Species as diverse as Artic charr (*Salvelinus alpinus*)(Olsen et al., 1998), house mice (Yamazaki et al., 1976; Eklund, 1997; Penn and Potts, 1998b) and humans (Ober et al., 1997; Wedekind and Furi, 1997) prefer to mate with individuals that have dissimilar MHC genes. These findings of disassortative mating are concurrent with theories of sexual selection emphasising the importance of immunocompetence (Jordan and Bruford, 1998). Research into MHC-mediated mate choice is adding to increasing evidence suggesting that females can increase the viability of their offspring by selectively mating with preferred males (Penn, 2002).

3.14 Olfactory Communication

Cats communicate using auditory, visual, tactile and olfactory signals. Indications of an animal's status could be conveyed via any of these signals. The Carnivora depend greatly on scent for communication (Gorman and Trowbridge, 1989) The majority of species belonging to Felidae are solitary living, and individuals are spatially scattered (Sandell, 1989). It is thought that *Felis silvestris libyca*, the ancester of the domestic cat, and many of the smaller species in the Felidae generally hold exclusive territories (Smithers, 1983; Bradshaw and Cameron-Beaumont, 2000). Solitary animals living in exclusive territories may only rarely encounter any conspecifics. When these solitary animals are efficient predators with the potential to inflict grievous bodily harm, the ability to communicate at a distance becomes an important component of an animal's repertoire (Bradshaw and Cameron-Beaumont, 2000).

The domestic cat has a large intraspecific variation in its spatial and social organisation, from very low densities and solitary lives through to very high densities and highly social lives (Calhoon and Hapel, 1989). Domestic cats frequently live at densities several magnitudes higher than the population densities of their wild ancestors, and as a result it is possible that aspects of their behaviour, including their olfactory communication has been changed over thousands of years of association with human culture.

The most obvious method of olfactory communication is urine marking. Cats can deposit urine in two methods. Urine may be sprayed when the cat backs up to a vertical object, while raising the tail between 45 and 90 degrees, and directing a fine spray of urine backwards, usually while quivering its tail (Natoli, 1985; Bradshaw and Cameron-Beaumont, 2000). This is determined as deliberate scent marking as opposed to excretory urine elimination, during which the cat squats to urinate and usually covers the urine with soil or litter (Bradshaw and Cameron-Beaumont, 2000). Urine marks sprayed by a conspecific may leave information about a trespasser presence inside a resident's territory and the time of their passing (Leyhausen, 1965). De Boer (1977) found that captive domestic cats showed greater interest, in terms of sniffing and flehmen, in fresh urine (30 minutes to 4 hours old) than they do in older urine (more than one day old). Natoli (1985) found that cats living in a free-ranging colony spent more time investigating the sprayed urine of unfamiliar males than the urine of familiar males. Furthermore, the duration of sniffing is inclined to increase with the unfamiliarity of the donating cat (Passanisi and Macdonald, 1990). All of these findings suggest that the intensity of the response is an attempt by the receiving cat to identify individual and temporal information in the olfactory message (Natoli, 1985; Bradshaw and Cameron-Beaumont, 2000).

During the oestrous season males display certain behaviours such as increased rubbing and urine spraying, the caterwauling courtship cry, courtship of oestrus females and mating (Yamane et al., 1994). It may be that the performance of these behaviours allows oestrous females to ascertain the calibre of each male. Much information is shared between cats using olfactory messages. Scent rubbing has been found to have both a calming and an orientation function (Pageat, 1996) however there has been little indication of its role in a sexual context. Males seem to be able to discern oestrus merely from the skin gland secretions of a female, but their interest is far greater when they are exposed to the urine of an oestrous female (Verberne and De Boer, 1976). Urine, particularly sprayed urine of both sexes, elicits more interest in both sexes.

3.15 Temperament

The terms individuality, personality and temperament are often used interchangeably in studies of animals (Mendl and Harcourt, 2000). There are many papers that contribute to the understanding of the development and stability of individuality in many animals, including humans (see review Mendl and Harcourt, 2000). Words such as temperament and personality are representative of the complex perceptions that humans have of a unique individual. Mendl and Harcourt (2000) describe such perceptions as " the sum total of all the behavioural attributes which characterise the individual and which distinguish it from others of the same species". The idea of the 'sum total' of an animal's behaviour is the result of a mental abstraction in the minds of the humans who interact with the animals over time, and who have made direct observations of the animals' behaviour in relation to that of others (Mendl and Harcourt, 2000).

Any pattern of behaviour may seem intangible when isolated events are considered separately, but collectively consistencies may emerge. People interacting with animals over a period of time may observe differences in the way that different individuals respond to a variety of circumstances and how they behave when interacting with other animals. Through these differences the observer may gain an insight into the overall pattern of an animals responses or what Feaver et al. (1986) termed their behavioural style. These general styles of response can then be described by adjectives such as 'friendly', 'bold', or 'nervous'. The weakness of such abstract assignation of individuality is that each observer may perceive something different based on the kinds of interactions each observer has had with the animal, the observer's experience of other animals with which to compare their behaviour, and other observer based bias (Mendl and Harcourt, 2000). Reassuringly, "the description of individual differences in a specifically defined behaviour pattern is likely to be achieved with reasonable agreement amongst observers"(Caro et al. 1979).

There are several papers on individual distinctiveness in cats (Feaver et al., 1986; McCune, 1995; Turner, 1999; Mendl and Harcourt, 2000) and other animals including brown bears (*Ursus arctos L.*) (Fagen and Fagen, 1996), black-tailed prairie dogs

(*Cynomys ludovicianus*) (Loughry and Lazari, 1994) and rhesus monkeys (*Macaca mulatta*) (Stevenson-Hinde and Zunz, 1978).

Feaver et al. (1986) assigned 18 separate behavioural categories to assess individual characteristics in the domestic cat. Three uncorrelated groups of cat behaviour emerged. Behaviour that was highly active and curious was categorised as 'alert'. Ratings of sociability with humans minus ratings of fearfulness and hostility to humans as well as tenseness formed the category 'sociable'. Cat behaviour that was relaxed and not easily disturbed was rated as 'equable'. Adult female cats living communally in a laboratory colony were then assessed using the three categories 'alert', 'sociable' and 'equable'. Females then fell into three further groupings. Cats that were scored positively 'alert' and 'sociable' and negatively 'equable' were active, aggressive cats that frequently gave threat and rarely retreated. Cats that scored poorly on all three categories were nervous and shy and frequently retreated from threats received. Cats that scored positively on all three categories were highly sociable, confident and relaxed animals that rarely gave or received threats (Feaver et al., 1986).

Even though distinctive modes of behaviour can be discerned among individuals, these need not be consistent over time. Behavioural styles appear to be reasonably consistent over time in some animals, including the cat (Cook and Bradshaw, 1995; Durr and Smith, 1997), although there is some anecdotal evidence suggesting unpredictable changes in certain individuals may occur due to changes in circumstance (Feaver et al., 1986).

3.16 Aims

The possibility of using conspecific male urine as an olfactory environmental enrichment technique for oestrous female domestic cats is investigated. Do oestrous females discriminate between individual males as an indicator of female preference for mates, and does the level of female relatedness to a male affect female preference? Eighteen oestrous females are presented with urine samples from four entire male domestic cats, and their responses in terms of sniff duration, latency, flehmen response and number of visits are recorded as measures of female interest. The females are placed alone in an observation room that is empty apart from the urine samples. Separation from pen-mates should maximise individual responsiveness and be more representative of the behaviour of socially isolated captive animals. The impact of individual temperament on female responses to urine is also investigated.

3.2 Materials and Methods

The response of eighteen entire female domestic cats (*Felis catus*) to the urine of four entire male domestic cats was investigated at the Heinz Watties Feline Nutrition Research Unit, a research unit of Massey University, Palmerston North, New Zealand (see Chapter 2). Observations were performed between January 2000 and November 2001. The management of the unit is described in Chapter 2.

3.21 Animals

All four entire males at the Feline Nutrition Unit were included in this study (Table 3.1). Three of these are potential breeding males and one, Bransen, has been kept entire for research purposes. Initially all the males were housed together in pen 14 but due to aggression between the two more dominant males, Sunny and Brock, Sunny, Titan and Bransen were moved to pen 11, while Brock remained alone in pen 14.

Table 3.1. The names, age, mating experience, pen number and the dominance rank of the males in this study.

Name	Born	Unit Bred	Mating Experience	Pen	Dominance Rank
Sunny	09/01/93	yes	yes	11	1
Bransen	09/02/97	yes	no	11	4
Titan	14/01/98	no	yes	11	3
Brock	14/01/98	no	no	14	2

The males, Brock and Titan, and females, Dusky, Sheba and Wren were brought as kittens to the unit in order to introduce new genetic stock in 1998.

The relative dominance status of each male has been established (see Chapter two, Table 2.2).

Eighteen entire females, from three separate pens were included in this trial (Table 3.2). None of these females were involved in any other study or palatability tests during the course of this project. Eight females were from pen 13, the breeding group. Four females from this group already had at least one litter, and the entire group has had prior experience with breeding males. The ten remaining females come from two separate groups of eight or nine cats that include a roughly equal number of neutered males. All the females are between two and eight years of age and regularly come into oestrus.

Table 3.2. The names, date of birth, source, number of litters and prior mating experience of the study females by pen.

Name	Born	Unit Bred	Litters	Mating Experience	Pen
Nikki	15/01/93	yes	3	yes	13
Fya	14/01/96	yes	1	yes	13
Kola	14/01/96	yes	1*	yes	13
Tori	24/0196	yes	1*	yes	13
Sheba	09/01/98	no	1	yes	13
Wren	16/01/98	no	0	yes	13
Dusky	05/01/98	no	0	yes	13
Bella	26/01/98	yes	0	yes	13
Nui	14/01/96	yes	0	no	10
Ziggy	04/01/97	yes	0	no	10
Pippe	05/01/97	yes	0	no	10
Asia	13/01/98	yes	0	no	10
Chyna	21/01/98	yes	0	no	10
Astra	13/01/98	yes	0	no	10
Ngio	09/02/97	yes	0	no	8
Broom	05/0197	yes	0	no	8
Muffy	04/01/97	yes	0	no	8
Suede	02/01/97	yes	0	no	8

*Kola and Tori each carried one litter full term but the kittens were dead at birth.

3.22 Relatedness

Details of the establishment of relatedness between males and females are provided in Chapter 2. Two of the males, Brock and Titan are recent additions to the unit and are unrelated to any of the females. The males Sunny and Bransen share varying degrees of relatedness with the females in this study (Table 3.3).

Table 3.3. The additive relationship between the females in this study and the unit-bred males Sunny and Bransen.

Females	Sunny	Bransen
Dusky	0	0
Fya	0.5	0.3125
Sheba	0	0
Bella	0.625	0.375
Nikki	0.25	0.75
Kola	0.5	0.3125
Wren	0	0
Tori	0.625	0.375
Nui	0.375	0.625
Chyna	0.625	0.5625
Muffy	0.25	0.5
Broom	0.375	0.5625
Suede	0.5	0.1875
Astra	0.5	0.1875
Pippe	0.375	0.5625
Ziggy	0.25	0.5
Asia	0.5	0.1875
Ngaio	0.375	0.75

3.23 Habituation

All of the females were habituated to the observation room and to a new person handling them. Eight of the cats were part of the study on the behaviour of anoestrus females in response to the urine of male cats (chapter 2). These females were already habituated to the observation room during that study and were familiar with the researcher. Ten female cats were new to this study and had to be familiarized with the observation room and with the new person handling them.

For three weeks the females were taken into the observation room so that they could become accustomed to being alone inside this new room. Initially they were cuddled and played with in groups of two and then on their own. They were then gradually left alone for longer and longer periods of time until they remained calm alone in the room for three minutes. These new females were not used in trials until February.

3.24 Oestrus

All of the females used in this study were known to regularly come into oestrus. For the purposes of this study behavioural correlates were used to determine the presence of oestrus. Females in oestrus will perform lordosis, where the female poses crouching low to the ground with her spine curved slightly ventrally and her tail laterally displaced. Many females will also issue 'heat' cries, long distinctive calls only performed during oestrus. When a female was observed to perform lordosis it was assumed that she was sexually receptive. Some females did not show obvious behavioural changes when in oestrus. For these individual cats, staff members at the unit confirmed oestrus as they were more familiar with the behaviour of each individual. Once the presence of oestrus was established each female's response to the urine of the entire males in the observation room was recorded. Testing continued until the female no longer performed lordosis and oestrus had presumably ended. The dates that oestrus was observed in each of the females were recorded (Appendix 3).

3.25 Experimental Design

The observation room and urine collections from male cats are described in chapter two. The urine of the four entire males was collected and placed individually on thick sheets of paper placed inside the observation room. When a female came into oestrus her response to the urine of the entire males was observed. Observations were carried out between 10am and 1pm. After 1pm the cats tended to be napping and settled for the afternoon. Before 10am there was considerable distraction for the cats caused by the routine caretaking at the unit.

Four regular sites around the observation room were selected and the urine of each male was randomly rotated between these four sites. This was to ensure that the visual cues for the females in the observation room remained the same, although the odours randomly changed. Once each male's urine had been removed from the fridge it was placed in its designated area in the observation room. Each urine sample was placed on two sheets thickness of 'Reflex' A4 paper (SCT Enhanced Performance, Australian Paper, 307 Mt.

Ferntree Gully Rd., Mt. Waverly 3149, Australia). The double layers of paper allowed easy disposal of the urine after the observations were complete and prevented the urine from sitting directly on the vinyl, thereby lessening the cross-contamination of odour between trials.

Once the urine had been placed for that particular trial, the observations began. Each female that was in oestrus on that day was put into the observation room one at a time. Once the cat was put through the cat-flap the stopwatch was started and the observations began. The cat's behaviour was recorded for three minutes and then she was returned to her home pen. Behaviour was recorded in real time and on videotape to ensure that no behaviours were missed.

Several specific aspects of the animals' behaviour were recorded (see Table 2.5, chapter 2) along with any other behaviours performed during the three minutes of observation time. Oestrous females were usually exposed to two or three combinations of the four urine samples on the days they were in oestrus, with an interval of at least one hour between exposures. The number of combinations depended on how many females were in oestrus on any given day, as few cats took less time and more combinations of the males' urine could be given to them. When one trial had finished the floor was thoroughly wiped down with water in an effort to remove any lingering odours before the next trial with the next combination of randomly placed male urine. At the end of the day any urine that had transferred to the floor, or been sprayed by the females, was sprayed with 'Pet S.O.X. Animaux' (Rolf C. Hagen Inc., Montreal, Quebec H4R 1E8) which removes all animal odours. The area was then washed down with water again the next morning before beginning the next day's trials. The cats were given lots of cuddles both before and after their trip to the observation room to ensure that they did not become stressed and the experience was an acceptable one.

3.26 Curiosity

There can be substantial differences in the levels with which each female responds. These differences can be unique and consistent within each individual female (see chapter two), however this could be confounded with the relatedness of the females to the males. Therefore, each female's responses towards Titan and Brock, the two males who are not related to any of the females, were used as controls for each individual female's responses towards the males to whom they are related. Each of the parameters that were recorded, sniff duration, latency, number of flehmen responses and number of visits, were used to show any consistencies in the way a female responded to the urine samples.

Where the female responses to all four males are compared, all males must be included in the analysis, so the female response to Titan and Brock could no longer be used as a control. The variations between female responses are compared to determine whether there are significant differences in the levels of curiosity with which each female responds to the urine samples. The level of female responsiveness is assessed using each parameter as an indication of the level of female curiosity.

3.28 Temperament

Three staff from the unit and HR assessed each cat according to the three main temperaments, 'confident' 'active' and 'nervous' from Feaver et al. (1986). Each person assessed the cats independently and temperaments were allocated to each cat based on the most common temperament assigned to that individual by the humans. Where there was a tie in the assessments of temperament for a particular cat, HR decided on the temperament to be allocated. A chi-squared test and a one-way ANOVA were used to analyse the effects of temperament on mate choice and curiosity.

3.29 Analysis

The data was analysed using analysis of co-variance (ANCOVA) in Systat Version 8. Two different models were used.

The data from the females' responses to the urine samples was analysed using all four males and focused on the female response to the males. The first model fitted included factors for males and females and used relatedness as a covariate. Females are included in the model as a block factor in order to account for the different degrees of interest that might be shown between the females.

A square root transformation was used for the sniff duration data and the data for latency to approach the urine samples was log transformed as the residuals were positively skewed and the variance of the residuals increased with increasing predicted values. If a female did not visit a urine sample within 3 minutes, latency is undefined and the cases were excluded form the analyses.

The second model focused on determining whether a female's relatedness to a male affects her behaviour. This model used 'males' (Sunny and Bransen) as factors. Relatedness and each female's level of curiosity as reflected by her response to the two unrelated males were the co-variates.

3.3 Results

On being placed in the observation room the queens responded by approaching one or more of the urine samples and sniffing. Once the urine had been investigated the cats generally did several things. They sniffed around the room, usually along the walls and around the door before sitting in front of the door and crying repeatedly until their time in the room was up. Some females went straight to the door and sat beside the door, either crying or being silent, until the observation period was finished. Occasionally a female would return to the urine of one or more males and sniff it again after they had investigated the room or even been sitting by the door. At the end of each observation period, females were usually sitting by the door.

Some females behaved in a different way each time they were placed in the room whilst others behaved in a relatively consistent manner. Some behaviours were unique to a particular cat. For example Nikki generally approached the urine of a male within the first 30 seconds. She invariably sniffed the urine of two or more of the males, frequently sniffing the urine of all four males, before sitting slightly back from the door and waiting silently until she was let out. Bella was the only cat to try to get out through the cat door.

Towards the end of two consecutive observation sessions Bella tried to open the cat door, but as the door was closed tight she eventually lost interest. Wren frequently spent some time rolling on the vinyl floor and Sheba chased dust motes and once chased the paper with the urine of the males on it around in a circle.

Dusky was the only female to urine spray during this study. When spraying occurred it usually followed the investigation of the males' urine. Dusky would then back up to the wall in her 'favoured' corner and spray urine. All but once she chose the same corner to spray in. Grooming always followed urine spraying. Dusky sprayed in eight out of the sixteen trials. When she did not spray she generally spent some time sniffing in the corner where she had previously sprayed, sometimes only an hour before. When Dusky sprayed, the urine was immediately cleaned up with clean towels and water but the smell persevered even after being sprayed with S.O.X. animal deodorizer.

The smell of another female's urine spray appeared to be of interest to most of the females who entered the room afterwards. Females sniffed at Dusky's spray for between 4 seconds and 63 seconds and a mean of 17.87 seconds, substantially longer than the mean sniff duration for any of the male urine.

The females also spent considerable time sniffing around the observation room. Occasionally one area of wall, or floor would become of sudden interest for no obvious reason, though once thoroughly sniffed by one female, others would also investigate this area.

There were other distractions from time to time such as spiders, cats in the playroom next-door, sounds from the general running of the feline unit. These noises attracted the cats' attention momentarily. Bella received a fright when a vacuum cleaner was turned on just as was being returned to her pen. After that she was very difficult to handle and scared of the observation room. She also hid in the rafters of her pen every time the vacuum cleaner was turned on for several months afterwards.

The latency to sniffing, duration of sniffing, ear and tail position were recorded. All other behaviours, including batting the paper, play, or urine spraying were at very low frequencies, or performed by only one female, and were not further analysed.

3.31 Habituation

During the period of habituating the cats to the new room several things were noted that contributed to the timing of the project.

If a cat had to be woken up to go into the observation room it became more agitated than it did otherwise and did not show any interest in anything other than returning immediately to its own pen.

The cat food is delivered on a big trolley holding trays of food for several pens. The cats become quite excited as the time or the trolley gets nearer. Cats that were taken to the room before they had got their new food could not be persuaded to play or be petted. Usually the cats just sat looking at the door.

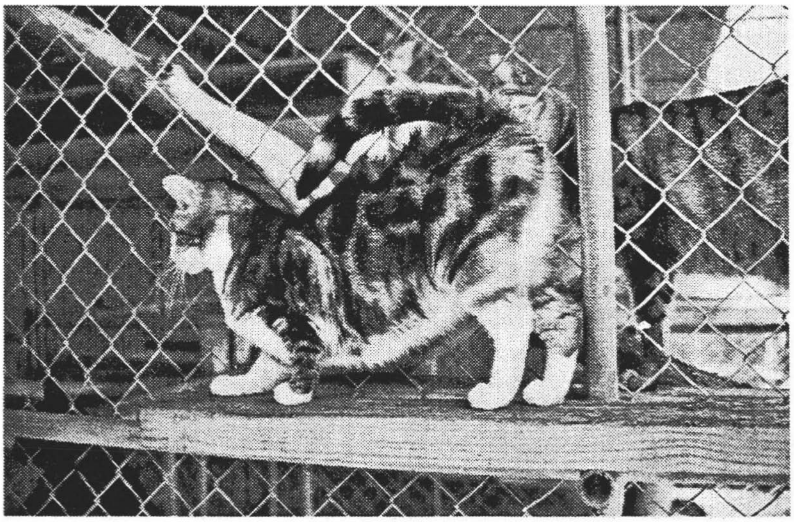
The length of the observation period was initially five minutes long, but the cats got upset once their interest was sated and they had to remain in the observation room until the five minutes was up. Therefore the time in the observation room was reduced to three minutes as all the queens had finished investigating by this time during the anoestrus trial.

3.32 Oestrus

Oestrus was clearly displayed in some females who performed lordosis, called, rolled on their backs and became extremely friendly. Other females behaved much as usual and oestrus was difficult to detect in these individuals. Staff members at the Feline Unit confirmed the presence of oestrus in females that were difficult to identify. Although all cats were observed in the morning to determine their state of fertility, cats that appeared not to be in oestrus were sometimes seen displaying the behaviours associated with oestrus later in the day when they were not being directly observed. Oestrus was frequently noted while the cats were in the recreation room for their regular play and weighing session. Cats were only included in the trial when oestrus had been firmly established using repeated observations of the behaviours associated with oestrus.

The onset of oestrus may have been missed in some cats during some cycles and in the cats that were particularly non-demonstrative an oestrus period may have passed by unrecognised. Oestrus lasted up to ten days and females were in anoestrus for about 10 days or longer (Appendix 2). Observations were taken over two or more oestrus cycles. Oestrus cycles of different females overlapped but appeared not to be strictly synchronous in any one pen, although due to the difficulty of determining oestrus in some individuals synchronicity may have been missed.

Oestrus became infrequent after May, but occasionally females have been observed by staff to still be cycling through winter. There is an increase in oestrus in spring. The study females were placed in with the males during September and October of 2000 and 2001, as the Feline Unit required kittens. For this reason the females were not tested until January 2001.



Bella performing lordosis, a behaviour associate with oestrus.

3.33 Dominance

The dominance ranks of the male cats were assessed previously (chapter two) but in addition the weights of each male during this trial agreed with the staffs' assessment of social dominance amongst the four cats (Table 3.5). Sunny was estimated as the most dominant male, with Brock next in ranking and constantly challenging Sunny for dominance. Titan was seen as third in the hierarchy and Bransen was the most subordinate male. This ranking order is reflected in the initial weights of the male cats. Sunny was the heaviest and weight decreased with social rank down to Bransen who was the lightest male (Table 3.5).

During January and February fighting between the dominant male Sunny and the challenging male Brock escalated, resulting in both males receiving injuries. After a particularly bad fight in which Brock was seriously injured, the males were separated into different pens. Brock had also been observed directing his aggressive behaviour towards the lower ranking cats, particularly the subordinate male Bransen. Brock was placed into a pen on his own while the remaining three males stayed together. This may have confused the ranking between of males. After separation the weight of all the males increased, however Brock's weight increased the most having no competition at the food bowl (Table 3.6).

3.34 Body Language

The position of each female cat's ear and tail were recorded as they approached the urine. The ears were consistently held sideways and slightly downwards, which denotes a submissive stance (Bradshaw, 1992). The tail was held low and straight. Occasionally the tail was tucked around the body once the cat had approached the urine sample and was sniffing.

3.35 Sniff Duration in Response to Male Dominance Rank

All of the females approached each of the males' urine and investigated by sniffing on a regular basis. Occasionally a female would sniff none of the urine samples during an observation period. All of the females sniffed each male's urine more than once over the duration of the study.

Table 3.5. The order of social dominance rank as assessed by staff members at the Feline Unit followed by their individual weights in grams as recorded over the period of the study.

Male	Sunny	Brock	Titan	Bransen
Assessed rank	1	2	3	4
Before Separation				
26/05/00	4468	4280	4280	4103
16/06/00	4732	4292	4202	4156
28/07/00	4750	4180	4132	4010
22/09/00	4291	4010	3697	3488
12/10/00	4300	3882	3581	3500
18/11/00	4114	4048	3522	3306
After Separation				
18/02/01	4110	4350	3710	3600
18/03/01	4140	4357	3641	3781
19/4/01	4203	4540	3972	4140
21/05/01	4287	4800	4034	4139

Table 3.6. Ranking from Tukeys multiple pairwise comparison. Sunny is significantly more interesting than marked* males. Remaining males are not significantly different from each other.

Rank	Sniff	Visits	Latency	Flehmen
1	Sunny	Sunny	Sunny	Sunny
2	Brock	Titan	Brock	Brock*
3	Titan*	Bransen*	Titan	Bransen*
4	Bransen*	Brock*	Bransen	Titan*

When analysing the responses to all four males, there was a strong female effect (Table 3.7) indicating that there are significant differences in the intensity of the response among the females (Figure 3.1).

A significant male effect was found, but there is no significant effect of relationship on the female response (Table 3.7, Figure 3.2).

Tukeys test was used to determine the order of preference between the males (Table 3.6). Sunny was sniffed longer than the other males. This difference was significant for Bransen (*P*=0.0001) and not quite significant for Titan (*P*=0.053). The difference between Sunny and Brock was not significant (*P*= 0.053).

3.36 Latency in Response to Male Dominance Rank

When those occasions when a urine sample was not approached were removed from the data set a considerable shift in the data occurred. The approach times evened out considerably (Figure 3.3), with all the males appearing to elicit similar approach times.

A significant female effect (Table 3.7) was found, indicating that there is a difference among the female responses. There is no significant effect for males, or for relatedness (Table 3.7).

No significant differences were found between the responses to the four males using the Tukeys multiple pairwise comparisons (Table 3.6).

3.37 Visits in Response to Male Dominance Rank

There is a significant difference in the responses towards the urine samples of the different males in terms of the number of visits each male's urine received from the females (Table 3.7, Figure 3.4). Tukeys multiple pairwise comparison of males revealed a significant difference between Sunny and Brock (*P*=0.033) and Sunny and Bransen (*P*=0.034). The difference between Sunny and Titan was not significant (*P*=0.066, Table 3.6)

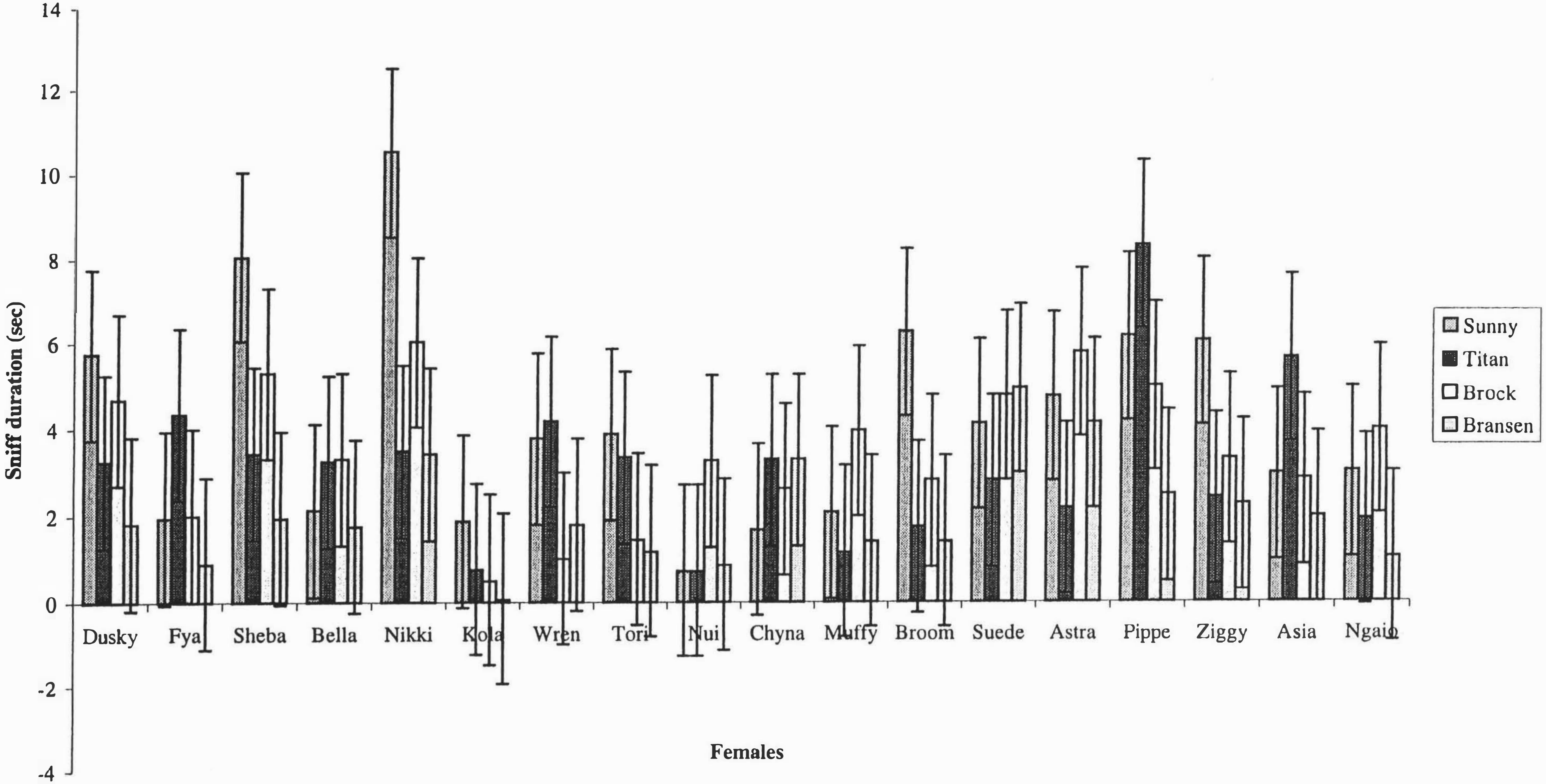
Table 3.7. The F-ratio and the p-value for the factors (males and females) and the covariate (relatedness) for each of the parameters of female behaviour that were recorded in response to the urine of all four males.

Parameter	Males		Female		Relationship	
Measured	$F_{3,50}$	P	$F_{17,50}$	P	$F_{1,50}$	P
Sniff Duration	7.72	<0.001	3.94	<0.001	2.82	0.100
Latency	2.33	0.007	2.45	0.007	2.88	0.095
Visits	4.02	<0.001	4.53	<0.001	2.68	0.108

Table 3.8. The F-ratio and p-value for the factor (males) and the covariates (relatedness and curiosity) for each of the parameters of female behaviour that were recorded in response to the urine of the two males that share varying degrees of relatedness with the females.

Parameter	Relatedness		Males		Curiosity		Interaction (Males*Relatedness)	
Measured	$F_{1,31}$	P	$F_{1,31}$	P	$F_{1,31}$	P	$F_{1,31}$	P
Sniff Duration	5.17	0.030	15.00	<0.001	12.82	0.001	4.33	0.046
Latency	0.11	0.747	0.003	0.96	0.01	0.929	0.01	0.905
Visits	9.55	0.004	20.03	<0.001	18.96	<0.001	13.01	<0.001
Flehmen	5.57	0.025	6.61	0.015	11.07	0.002	0.88	0.355

Figure 3.1. The average duration of sniffing by each female for each of the males



The female effect is significant in terms of the number of visits (Table 3.7) showing that the interest expressed by each female in terms of her inclination to visit a urine sample shows significant differences between the females.

There is no effect of relatedness on the female response in terms of visits when all four males are compared.

3.38 Flehmen Response to Male Dominance Rank

The number of flehmen responses observed is likely to be less reliable in this study than in chapter two due to the camera angle that allowed a full view of the observation room, but not a close view of the cats as they investigated the urine samples.

Flehmen was observed occasionally when the females' heads were raised, and although flehmen could not be observed every time it still appears to follow the same trend as the other parameters (Figure 3.5).

Because of the many non-responses, transformation to achieve normally distributed results was not possible. The flehmen data was converted to a binary variable and a logistic regression model was attempted but this did not work (maximum likelihood failed to converge) probably due to the large number of non-responses e.g. one male (Bransen) and eight females had no positive responses (Table 3.9 and 3.10).

A chi squared test showed significant difference in the probability of a flehmen response to the different males (χ^2 =10.9, df= 3, P= 0.012) but this does not take account of the different females or the effect of relatedness.

The Tukeys multiple pairwise comparisons showed a significant difference between Sunny and all of the other males (Bransen P= 0.001; Titan P=0.001; Brock P= 0.028) but these results are unreliable due to the many non-responses.

Figure 3.2. Mean female sniff duration for the urine of each male.

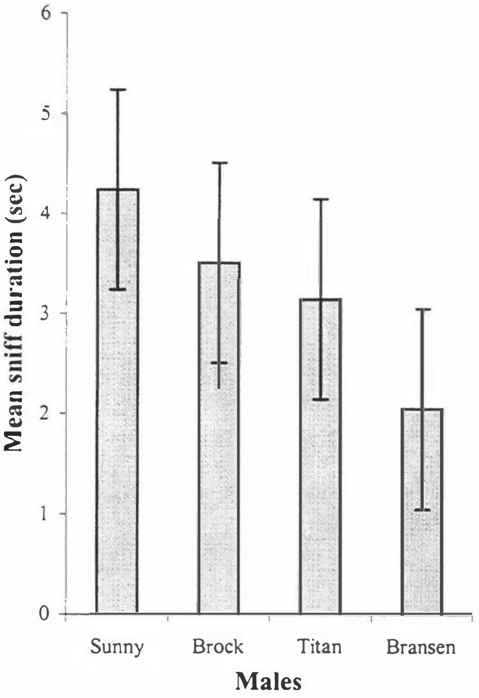


Figure 3.3. The overall mean latency to the urine samples of each male excluding occations when the urine was not approached.

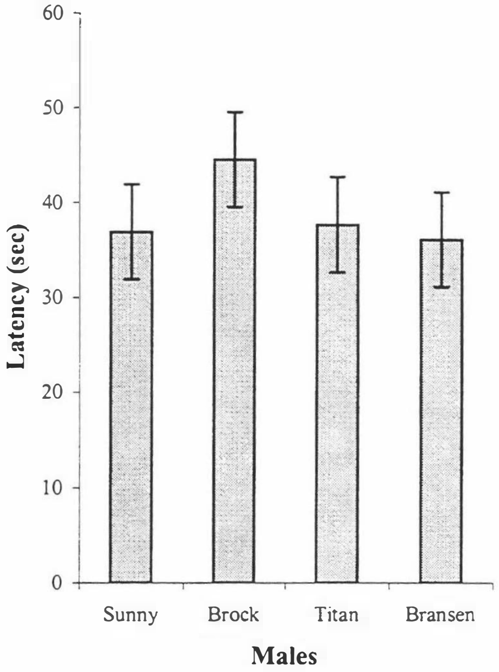


Figure 3.4. Mean number of female visits to the urine of each male.

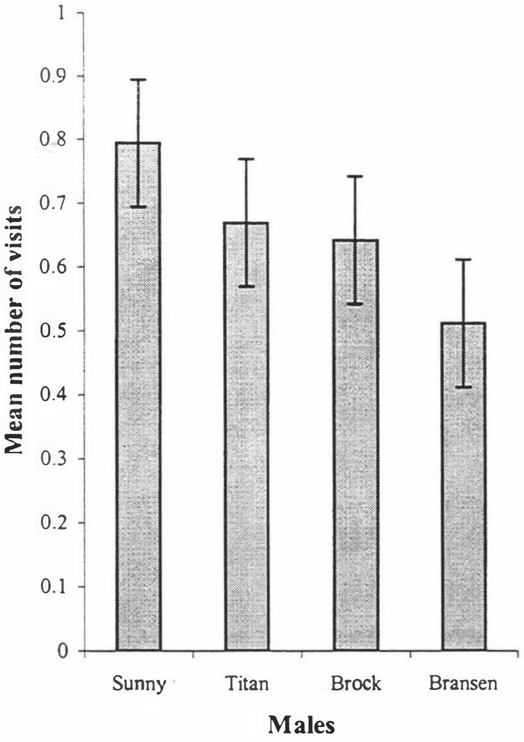


Figure 3.5. Average number of flehmen observed as response by females to the urine of each male.

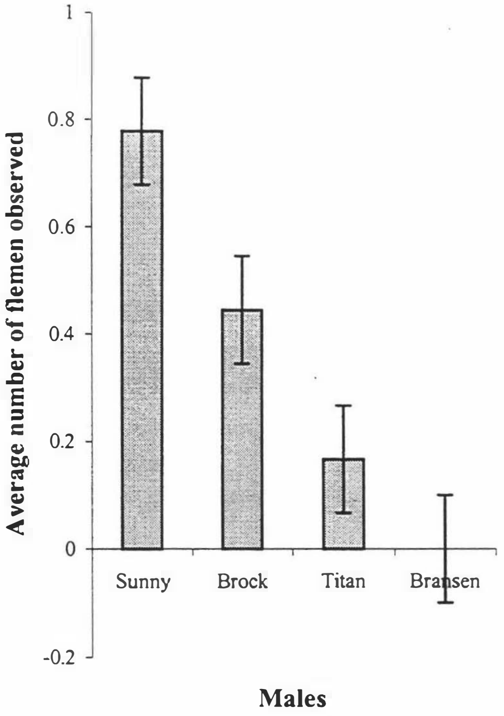


Table 3.9. Number of females that performed or did not perform flehmen in response to the urine of each male

Males	Number of Females		
	Flehmen	None	Total
sunny	14	4	18
brock	8	10	18
titan	3	15	18
bransen	0	18	18
Total	16	56	72

Table 3.10. Total number of flehmen observed by each female in response to the urine of each male

Males	Total number of female flehmen observed					
	Dusky	Fya	Sheba	Bella	Nikki	Kola
Sunny	1	1	2	0	4	0
Brock	0	0	1	0	3	0
Titan	0	1	0	1	0	0
Brock	0	0	1	0	3	0
Bransen	0	0	0	0	0	0
	Wren	Tori	Nui	Chyna	Muffy	Broom
Sunny	0	0	0	0	1	0
Brock	0	0	0	0	2	0
Titan	0	0	0	0	0	0
Bransen	0	0	0	0	0	0
	Suede	Astra	Pippe	Ziggy	Asia	Ngaio
Sunny	0	2	2	1	0	0
Brock	0	0	1	0	0	1
Titan	0	0	1	0	0	0
Bransen	0	0	0	0	0	0

3.39 Ranking of the Males Summarized

Figures 3.3, 3.4, 3.5 and 3.6 show a pattern that is consistent with the dominance rank assigned to the males (Table 3.5.). Sunny, the most dominant male received the most interest overall. Bransen, the most submissive male received the least attention overall, and Titan and Brock are intermediate (Table 3.6).

3.310 The Effect of Relatedness on the Female Sniff Duration

The relatedness of Sunny and Bransen to each of the females, varied from between 0.0 (unrelated) to 0.75 (highly related).

The effect of relatedness on sniff duration was found to be significant (Table 3.8), with the sniff duration lessening as the degree of relatedness increased (Figure 3.6).

A significant male effect was found between the responses, with females sniffing longer overall for Sunny than they did for Bransen (Table 3.8) regardless of how related they are to either male (Figure 3.6).

There was also a significant interaction between the effect of relatedness and the two males (Table 3.8) with Sunny showing a stronger interaction (steeper slope) than Bransen (Figure 3.6). Females that were unrelated to Sunny tended to sniff his urine longer, and the sniff duration drops off as the degree of relatedness increases. Although females that are highly related to Bransen do not sniff his urine for very long, females that are unrelated do not appear to sniff Bransen for a longer duration. This indicates that even when the females are unrelated to the males, Sunny is found to be a more interesting male to smell than Bransen. The lines intersect each other (Figure 3.6), but Sunny's highest additive relatedness is 0.625, whereas Bransen's highest additive relatedness is 0.75 and the lines are an extrapolation.

The effect of curiosity in terms of mean sniff duration was found to be significant (Table 3.8), with females that generally had a longer sniff duration with the control males, also sniffing longer at both the males Sunny and Bransen (Figure 3.7).

3.311 The Effect of Relatedness on the Female Latency

No effect of relatedness was found, and no significant differences were found between the latency to either of the two males Sunny and Bransen (Table 3.8 and Figure 3.8). There was no effect of female curiosity (Table 3.8). How long the average female latency was to the unrelated males did not predict how long the latency to first sniff was among the individual females for related males Sunny and Bransen (Figure 3.9). A female's innate level of curiosity and her degree of relatedness to the males does not appear to influence how quickly a female will approach a urine sample. There is no male effect when the two related males are considered, and there wasn't any male effect for latency when all four males were considered (Tables 3.7 and 3.8).

3.312 The Effect of Relatedness on Visits

A significant effect of relatedness was found for visits (Table 3.8, Figure 3.10) for both Sunny and Bransen. Once again, there is a male effect with Sunny visited more than Bransen (Table 3.8, Figure 3.10), although the slope shown for Sunny is an extrapolation as none of the females were quite as highly related to him as they were to Bransen.

There was also an interaction between males and the degree of relatedness (Table 3.8), showing that the effect of relatedness is stronger for Sunny (steeper slope) than it is for Bransen (Figure 3.10). When the degree of relatedness is equally low Sunny is visited more than Bransen. This preference for Sunny drops sharply as the additive relatedness to a female increases (Figure 3.10).

The effect of female curiosity was found to be significant (Table 3.8) for females visiting the urine samples of Sunny and Bransen (Figure 3.11). Females that tended to visit urine samples more frequently for the control males also visited the urine samples of Sunny and Bransen more frequently.

Figure 3.6. Sniff duration plotted against the additive relatedness of each of the females to the males Sunny and Branson.

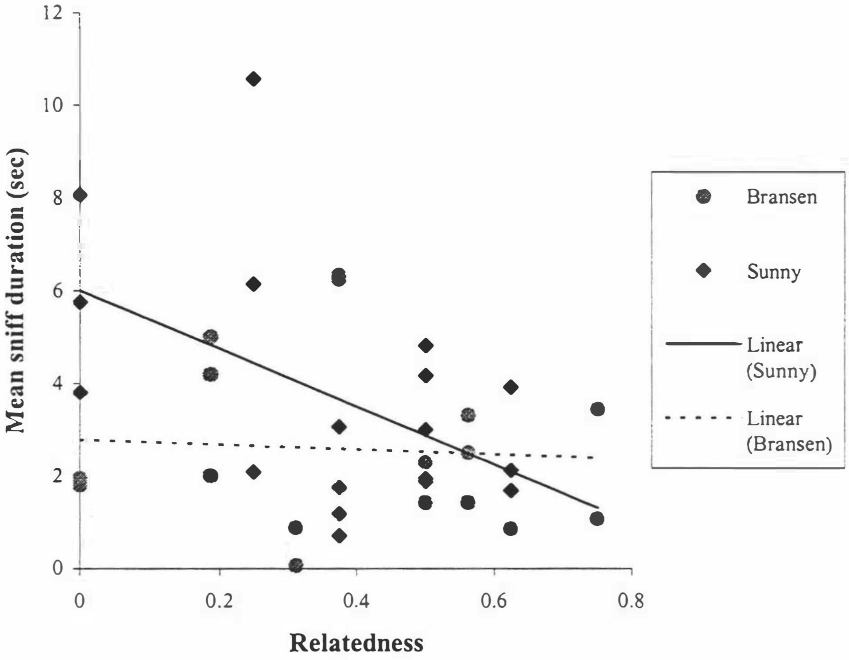


Figure 3.7. Mean sniff durationat the urine of the males Sunny and Bransen plotted against the curiosity of each female shown by their response to the control urine samples.

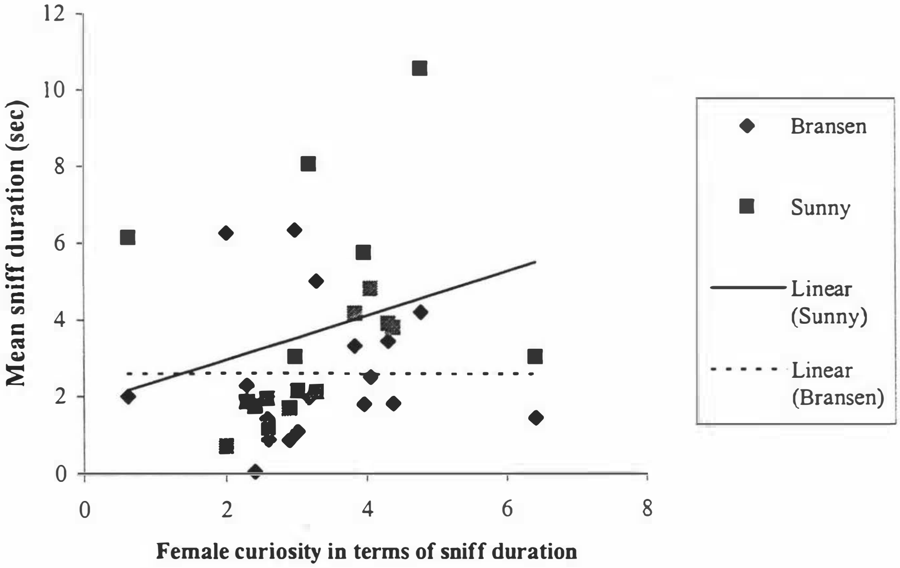


Figure 3.8. Female latency to approach the urine samples of Sunny and Bransen plotted against the relatedness of each female to those males.

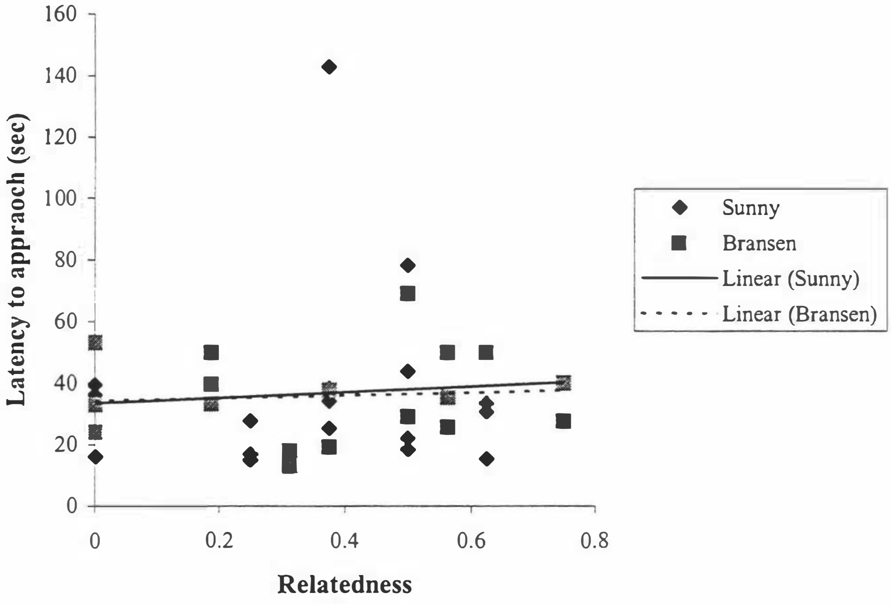
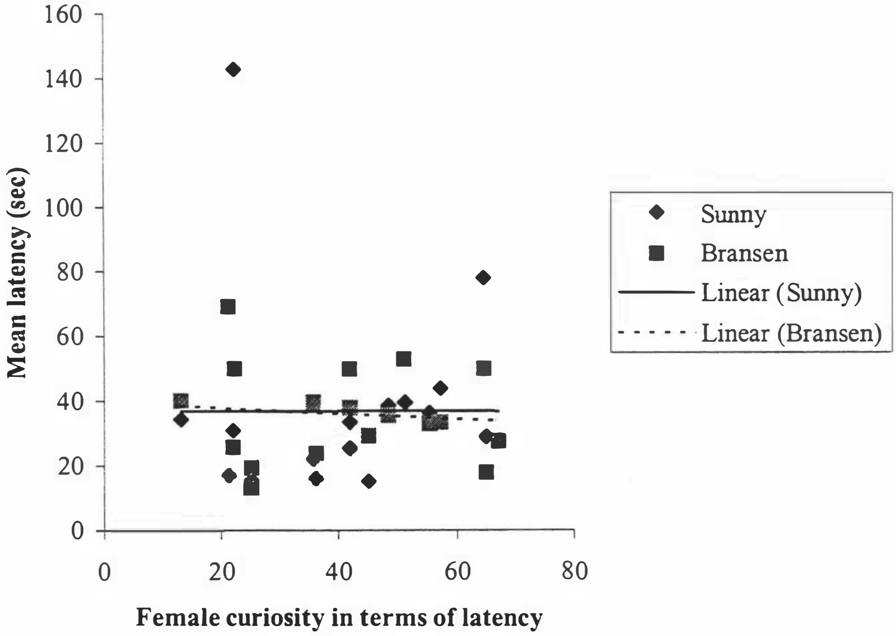


Figure 3.9. Female latency to approach the urine samples of the males Sunny and Bransen plotted against the curiosity of each female



3.313 The Effect of Relatedness on the Flehmen Response

The effects of relatedness and male were found to be significant (Table 3.8), with the Sunny receiving significantly more flehmen responses than Bransen (Figure 3.12).

There was no significant interaction between the responses by the females to the two different males Sunny and Bransen in terms the effects of the females' increasing relatedness of each male (Table 3.8). This may in part be due to lack of positive responses for this parameter. Many of the females were not observed to flehmen in response to any of the urine samples, and even females that did show this response might have only shown it once or twice. The female who showed the highest number of flehmen responses was Nikki with a total of seven, over the duration of the study (Table 3.10).

There was a significant effect of female curiosity (Table 3.8). Females that were found to express flehmen in response to the urine of unrelated males also tended to respond with flehmen towards related males (Figure 3.13). However, no females were observed showing the flehmen response to the urine of Bransen (Tables 3.9 and 3.10).

3.314 Curiosity and Temperament

The effect of each female's idiosyncrasies in terms of the curiosity she exhibited in the observation room was the most consistent results. The females behaved differently from each other, in terms of their general behaviour in response to the other females, to humans, to the observation room and in terms of the parameters recorded as a measure of their interest. Therefore this aspect of the females behaviour was looked at further.

Interestingly none of the cats was assigned more than two personality traits (Table 3.11), despite the acceptance that the three major personality traits, confident, nervous and active are acknowledged to be on a continuum.

Figure 3.10. Average number of visits to the urine samples plotted against the additive relatedness of each female to the males Sunny and Bransen.

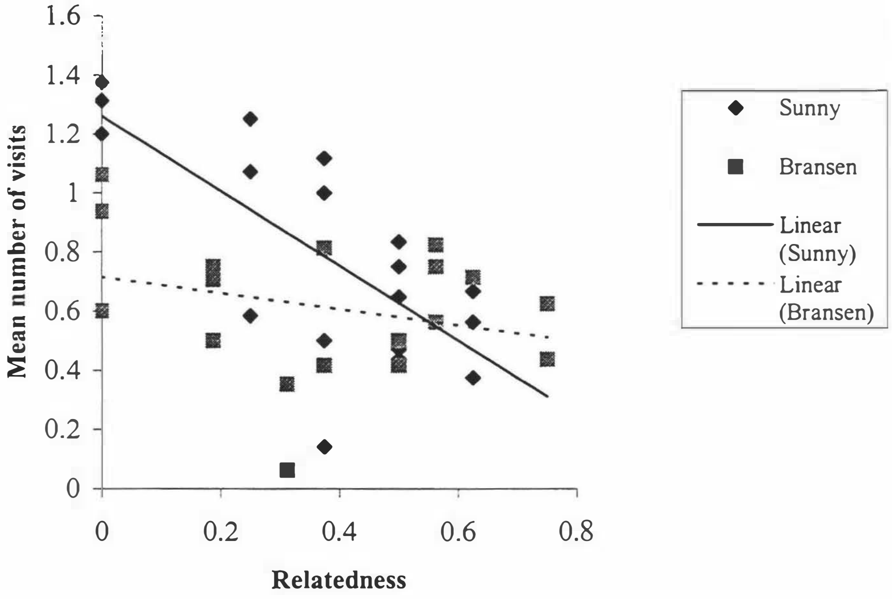


Figure 3.11. Average number of visits to the urine samples of Sunny and Bransen plotted against each female's overall curiosity in terms of visits.

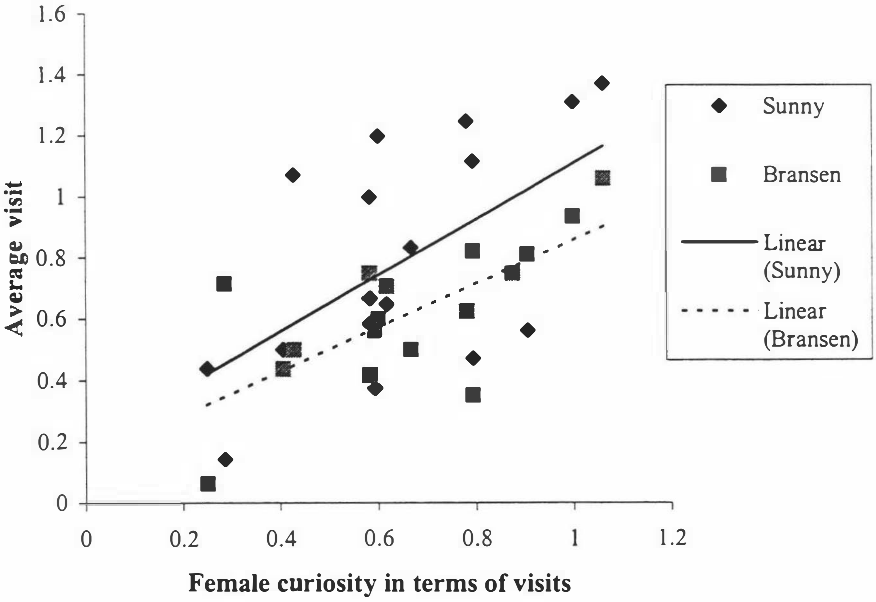


Figure 3.12. Average female flehmen in response to the urine of Sunny and Bransen plotted against the additive relatedness of the females to each male.

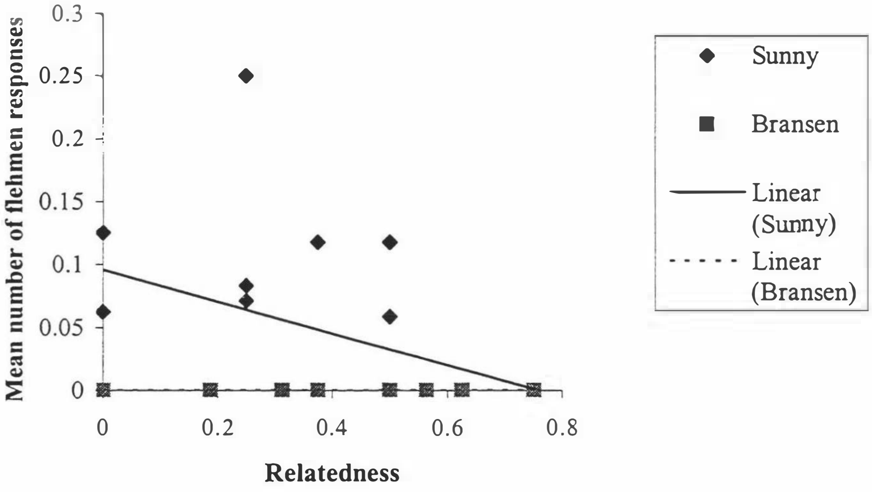
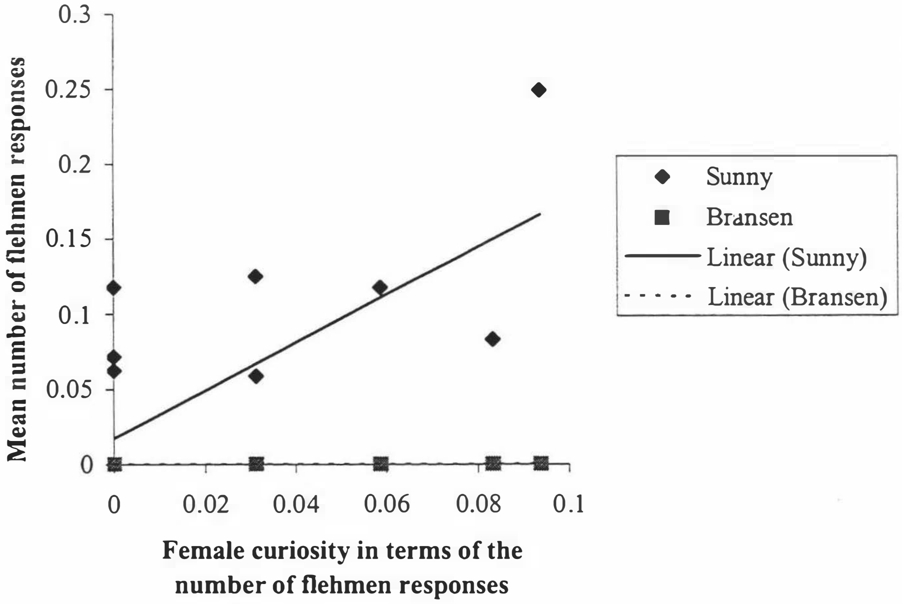


Figure 3.13. Average female flehmen in response to the urine of males Sunny and Bransen plotted against the average interested-ness of each female.



The cats are matched by female preference for the male urine and by temperament (Table 3.12). The matched temperaments show that most of the 'active' females appear to prefer the urine of the 'active' male Brock. Most of the 'confident' females appear to prefer the urine of 'confident' males Sunny and Titan. None of the 'nervous' females preferred the urine of the 'active male' Brock. This trend is not significant ($\chi^2 = 8$, df=4, $P=0.091$) but due to the small number of females available all expected frequencies are less than 5 so the test is not reliable (Table 3.13). Even when the two confident males are combined the result is still not significant ($\chi^2 = 4.8$, df=2, $P=0.091$) and most expected frequencies are still less than 5.

When the relatedness to Sunny is taken into account any trend for 'temperamental' preferences may be the result of female preferences for the urine of male to whom they are only slightly related. Females with an additive relationship to Sunny that is greater than 0.375 usually preferred one or other of the males, Titan and Brock, who are not related to any of the females. The exceptions are Kola and Tori who both have an additive relationship to Sunny that is higher than 0.5 (Table 3.12). Both of these females have had unsuccessful litters that were sired by Sunny.

However, some females appear to have preferences for one particular male's urine. The male that received the most attention in each parameter was listed to determine whether there were any trends in female preference (Table 3.14). The majority of significant results from the Tukeys tests (Table 3.6) showed a greater response to the urine of Sunny. However, some females appeared to be more interested in Brock or Titan (Figure 3.14), although there was insufficient data when the females are considered individually to determine whether there are any significant preferences. There was an interesting consistency in the preference for a particular male over the different parameters. Only six females showed a preference for different males between sniff duration and visits (Table 3.14). Latency has been included in the table even though it was not found to be a significant indicator of interest. There is however some consistency between latency and sniff and visits. Ten of the females that showed a consistent preference for a particular

Table 3.11. Individual cat's dominant temperatment as percieved by humans who work at the Feline Unit and eachs cat's overall domiant personality trait.

Female Cat	Heather	Karin	Rachael	Heidi	Temperatment
Nikki	confident	nervous	confident	confident	confident
Sheba	active	active	active	active	active
Dusky	nervous	nervous	nervous	nervous	nervous
Wren	active	active	confident	active	active
Bella	active	active	confident	active	active
Kola	nervous	nervous	nervous	nervous	nervous
Fya	nervous	nervous	nervous	nervous	nervous
Tori	confident	confident	active	confident	confident
Ngaio	active	confident	active	active	active
Nui	active	confident	confident	confident	confident
Asia	confident	confident	confident	confident	confident
Chyna	active	confident	confident	confident	confident
Astra	confident	confident	confident	active	confident
Ziggy	confident	active	confident	confident	confident
Suede	active	active	active	active	active
Broom	confident	active	confident	confident	confident
Pippe	nervous	nervous	confident	confident	confident
Muffy	active	confident	active	active	active
Male Cats					
Sunny	confident	confident	confident	confident	confident
Brock	active	active	active	active	active
Titan	confident	confident	confident	confident	confident
Bransen	nervous	nervous	nervous	nervous	nervous

Table 3.12. Prefered male and his temperament by the choosing female and her temperatment. The additive relatedness of each female to Sunny is included as relatedness was found to have a significant effect on female responses. Relatedness to Bransen is not listed as his urine was not prefered by any females

Prefered Male	Temperatment	Female	Temperatment	Relatedness to Sunny	
Brock	active	Bella	active	0.625	Bella
		Muffy	active	0.25	Muffy
		Suede	active	0.5	Suede
		Ngaio	active	0.375	Ngaio
		Astra	confident	0.5	Astra
		Nui	confident	0.375	Nui
Sunny	confident	Dusky	nervous	0	Dusky
		Kola	nervous	0.5	Kola
		Nikki	confident	0.25	Nikki
		Tori	confident	0.625	Tori
		Ziggy	confident	0.25	Ziggy
		Broom	confident	0.375	Broom
		Wren	active	0	Wren
		Sheba	active	0	Sheba
Titan	confident	Fya	nervous	0.5	Fya
		Pippe	confident	0.375	Pippe
		Asia	confident	0.5	Asia
		Chyna	confident	0.625	Chyna

Table 3.13. Summarised females temperament listed against their preferred male

	Female Temperament		
Preferred Male	Active	Confident	Nervous
Sunny	2	4	2
Brock	4	2	0
Titan	0	3	1

Table 3.14. Listing of the preferred male for each female as indicated by their sniff duration, latency and number of visits, followed by their additive realtionship to the two males bred at the Feline unit. Asterix following the female names indicates a significant preference for a particular male in terms of sniff duration. Consistent choices throughout each of the parameters have been highlighted, including those that are consistent only between sniff duration and visits as latency was not found to be a significant predictor of female interest.

Female	Preferred male			Additive relationship with Sunny and Bransen			
	Sniff duration	Latency	Visit	Relation	Sunny	Relation	Bransen
Dusky*	Sunny	Sunny	Sunny	0	Dusky	0	Dusky
Fya	Titan	Titan	Titan	0.5	Fya	0.3125	Fya
Sheba*	Sunny	Sunny	Sunny	0	Sheba	0	Sheba
Bella	Brock	Brock	Titan	0.625	Bella	0.375	Bella
Nikki*	Sunny	Sunny	Sunny	0.25	Nikki	0.75	Nikki
Kola	Sunny	Sunny	Sunny	0.5	Kola	0.3125	Kola
Wren	Titan	Sunny	Sunny	0	Wren	0	Wren
Tori	Sunny	Sunny	Sunny	0.625	Tori	0.375	Tori
Nui	Brock	Bransen	Bransen	0.375	Nui	0.625	Nui
Chyna	Titan	Bransen	Brock	0.625	Chyna	0.5625	Chyna
Muffy	Brock	Brock	Brock	0.25	Muffy	0.5	Muffy
Broom*	Sunny	Sunny	Sunny	0.375	Broom	0.5625	Broom
Suede	Bransen	Sunny	Sunny	0.5	Suede	0.1875	Suede
Astra*	Brock	Titan	Brock	0.5	Astra	0.1875	Astra
Pippe*	Titan	Titan	Sunny	0.375	Pippe	0.5625	Pippe
Ziggy*	Sunny	Sunny	Sunny	0.25	Ziggy	0.5	Ziggy
Asia	Titan	Bransen	Titan	0.5	Asia	0.1875	Asia
Ngaio	Brock	Brock	Brock	0.375	Ngaio	0.75	Ngaio

Table 3.15. Female temperament, matched with female curiosty as determined by each females mean number of visits and mean sniff duration in response to the males to whom she is not related.

Female	Curiosity (visits)	Temperament
Sheba	1.063	Active
Dusky	1.000	Nervous
Bella	0.906	Active
Asia	0.875	Confident
Fya	0.794	Nervous
Pippe	0.794	Confident
Nikki	0.781	Confident
Suede	0.667	Active
Astra	0.618	Confident
Wren	0.600	Active
Chyna	0.594	Confident
Muffy	0.583	Active
Broom	0.583	Confident
Tori	0.583	Confident
Ziggy	0.429	Confident
Ngaio	0.406	Active
Nui	0.286	Confident
Kola	0.250	Nervous

Female	Curiosity (sniffs)	Temperament
Pippe	6.412	Confident
Nikki	4.781	Confident
Sheba	4.375	Active
Asia	4.313	Confident
Astra	4.059	Confident
Dusky	3.969	Nervous
Suede	3.833	Active
Bella	3.281	Active
Fya	3.176	Nervous
Ngaio	3.000	Active
Chyna	2.969	Confident
Ziggy	2.893	Confident
Wren	2.600	Active
Muffy	2.583	Active
Tori	2.409	Confident
Broom	2.292	Confident
Nui	2.000	Confident
Kola	0.625	Nervous

male by having greater sniff duration and a greater number of visits and a shorter latency to approach the sample of that same male (Table 3.14).

Generally the females who preferred Sunny shared a lower level of relatedness with him than those females who preferred Titan or Brock (Table 3.14).

The assigned female temperament was also matched with each female's mean number of visits and mean sniff duration to the males, Titan and Brock, who were used as controls because they were unrelated to all of the females. These two parameters were chosen because they achieved the highest level of significance for the effect of female curiosity (Table 3.7 and 3.8). However, when this was analysed with a one-way ANOVA the results were not significant (sniffs: $F_{2,15}= 0.265$, $P=0.77$, visits: $F_{2,15}= 0.644$, $P=0.54$). There is no difference in curiosity among temperament categories (Table 3.15).

3.4 Discussion

Oestrous female domestic cats (*Felis catus*) were exposed to urine samples of four entire male cats and their responses in terms of interest were recorded. Four parameters were recorded to measure the interest of the females: sniff duration, visits, flehmen and latency to approach the urine samples.

- There was a significant difference in the female response towards the males Sunny and Bransen who shared varying degrees of relatedness with the females in this study.
- Overall interest shown to each male by the females appears to follow the dominance rank of the males, with Sunny the most dominant male receiving the most attention. The newly introduced males Brock and Titan held intermediate levels of interest for the queens and Bransen the most sub-ordinate male receiving the least attention from oestrous females. No significant difference was found between the female response to the unrelated males and the response toward the unit bred male Bransen.
- The degree of relatedness was correlated with the sniff duration, the number of visits and the number of flehmen shown in response to the urine of the males to whom the

oestrous females were related. The higher the degree of relatedness the shorter the sniff duration, the lower the frequency of visits and flehmen.

- The interaction between the males and the effect of relatedness showed that the relatedness effect was significantly stronger for Sunny, than it was for Bransen. Females that were unrelated, or only slightly related to Sunny found him much more interesting than females who were highly related to Sunny. By contrast, females that were unrelated or shared a low level of relatedness with Bransen did not find him quite as interesting, although lack of interest increased as female relatedness to Bransen increased.
- There is a significant difference among individual females in the level of interest shown to male urine. The unrelated males used as controls were found to be accurate predictors of female behaviour in terms of sniff duration, visits and flehmen. Females showed a significant consistency within individuals in their general level of interest for all parameters except latency.

3.41 Temperament

There were significant differences in the level of female curiosity for all parameters except latency, showing both that there were consistencies within a female cat and differences between the different females in their general level of interest. When the female response towards the urine of the two males that shared varying degrees of relatedness with the females were considered the two unrelated males were used as controls. Females, who sniffed longer at the urine of the unrelated males, also tended to sniff longer at the urine of related males. Females who visited the urine of unrelated males infrequently also tended to visit the urine of related males less frequently.

Because of the consistencies in the level of curiosity shown by each female three categories of temperament extended from Feaver et al. (1986) were assigned to the females and the males. There was a surprising consistency in the temperaments assigned to each cat. There was only one case where there was a tie between two temperaments assigned to one cat. Otherwise either there was unanimous, or majority agreement in the temperaments assigned to the cats by the observer and staff from the feline unit. No cat was assigned all three of the potential temperaments. There appears to be a perception that each cat had usually one predominant behavioural style, although this showed some overlap with one other style. Cats were seen to be either predominately active with confident or nervous tendencies, predominately confident with nervous tendencies or with active tendencies, or predominately nervous with confident tendencies or active tendencies. Even when the dominant behavioural style was so strong that all four people assigned the same trait, other tendencies can still be observed. An active, confident cat can still become nervous when placed in an upsetting situation.

The temperaments assigned are merely snap shots of each animal's behavioural style during the period of this study. Most cats' temperaments appear to be quite stable over time, as observed by feline unit staff (Karin Weidgraaf and Heather Nicol per communication) although, for the females, changes such as pen re-arrangements, giving birth and raising kittens, or increased handling can potentially cause changes in the predominant behavioural style.

The temperaments assigned to each female were then matched with their most preferred males assigned temperament. This did not show any significant correlation. Even if temperament were a factor that females take account of during mate choice, the varying degrees of relatedness between the cats in this study may obscure any female preference based on other factors. Each female's assigned temperament was then matched with the values obtained as measures of individual female curiosity in terms of sniff duration and visits. There was no significant correlation between the assigned temperament and female curiosity. Confident females are no more likely to be curious than active or nervous females.

3.42 Relatedness

The additive relatedness was calculated between each of the females and the two males bred at the feline unit. As the level of relatedness between the genders increased the interest shown by the oestrous female in the urine from the related male decreased. This phenomenon was strongly significant for sniff duration, visits and flehmen but not for latency. There was also a significantly different response between the two males. The relatedness effect appeared much stronger for Sunny, due to the fact that females with a low relatedness (0.25 or less) or no relatedness showed a higher level of interest in Sunny than they did in Bransen. Sunny's social dominance over Bransen may have influenced this response, or the fact that Bransen is slightly inbred with an additive relatedness to himself of 1.25 may have resulted in the reduced interest shown for him. Bransen also held slightly higher levels of shared relatedness with more of the females than Sunny did. This may have emphasised the trend showing that Bransen was not a particularly attractive male when compared to Sunny. The effect of relatedness was not detectable when all four males were compared because the other two males had zero relatedness to all of the females in this study.

The harmful effects of inbreeding in domestic cats in terms of fitness and survival independent from human care are as yet scientifically unidentified, but close kin matings have been frequently observed (Liberg et al., 2000). Indeed close kin matings are considered essential when developing and even maintaining pure 'breeds' of domestic cats (Wright and Walters, 1980). The subsequent mutations which are revealed by the recessive alleles that are no longer sheltered by heterozygosity, such as tail-lessness for the Manx, or hair-lessness for the Sphinx are considered desirable by humans, however such mutations would naturally confer a significant disadvantage on a feral cat. However when females are given the option they appear to attempt to avoid mating with close kin males (Ishida et al., 2001). The present study shows that urine scent is a possible way that females can make this assessment and choice.

Females living in groups with closely related males showed a greater tendency to leave their home group during oestrus than females who lived in groups without a male (Liberg, 1983). More compelling evidence of inbreeding avoidance in the domestic cat was demonstrated when female domestic cats in feral populations, were shown to avoid copulating with close kin males but not with distant kin males. Furthermore, females were less likely to accept copulations with any related males than they were with non-kin males (Ishida et al., 2001).

In this study oestrous females spent less time investigating the urine of a male to whom they are highly related, than the urine of a male to whom they are only distantly related, or unrelated. These results infer that an oestrous female domestic cat can determine the degree of relatedness she shares with a male from the smell of his urine.

3.43 Kin Recognition and the Major Histocompatibility Complex

Kin recognition has been recorded for a number of species, including humans (Motluk, 2002) and has been linked with recognition of the major histocompatibility complex through chemosensory mechanisms (Manning et al., 1992; Eggert et al., 1998a; Yamazaki et al., 2000). The major histocompatibility complex (MHC) genes are extremely polymorphic and play a significant role in the cellular immune response (Brown and Eklund, 1994; Penn and Potts, 1998a; 1999).

Selection based on inbreeding avoidance and mating preferences may help promote the high degree of heterozygosity within the loci associated with the MHC, and most likely reduce homozygosity throughout the genome (Potts and Wakeland, 1993; Eggert et al., 1998a). The offspring MHC disassortative matings would benefit from increased MHC heterozygosity inferring increased resistance to infectious disease as well as increased levels of fitness due to reduced levels of inbreeding depression (Potts and Wakeland, 1993; Penn and Potts 1999). However fitness benefits from inbreeding avoidance, and its associated increase in genome-wide heterozygosity, have been easier to detect than fitness benefits solely for MHC heterozygosity, suggesting that inbreeding avoidance presents the greatest potential benefits. Therefore by increasing the MHC heterozygosity or the genome wide heterozygosity, MHC-disassortative mating preferences should increase the genetic compatibility of mates (Penn, 2002).

3.44 Odour Cues

A considerable number of studies using rodents and a series of field studies with humans have revealed comprehensive picture of MHC-related odour signals and given validation to the theory of immunogenetic linked odortypes. (Eggert et al., 1998a)

Identification of MHC dissimilarities between the individuals in these studies was associated with olfactory cues, predominantly in urine (Eggert et al., 1998a; Yamazaki et al., 1998). The genes situated at the MHC are accountable for individual variation in body odour (odortypes). 'Odorants' or their precursors concentrate in urine after being bound directly by MHC products (Yamazaki et al. 1998). House mice that are identical except for the genes of the MHC have distinctive urinary odours that can be distinguished by conspecifics (Brown, 1995; Penn and Potts, 1998a).

However, the chemical cues involved with mammals outside of the laboratory are controversial and appear to involve micro-organisms (Brown and Eklund, 1994, Ehman and Scott, 2001) and diet (Schellinck et al., 1992). Non-MHC genes and the X and Y chromosomes also influence the urinary odours of rodents, and rearing rats in a bacteria free environment inhibits the manufacture of distinctive MHC- related odours." (Brown, 1995). Brown (1995) found that dietary differences have a greater effect on individually characteristic odours than differences at one MHC locus. These results indicate that the MHC, commensal bacteria and dietary products act together to generate urinary odours which can be used for individual recognition in rodents (Brown, 1995).

3.45 Attractive traits

The dominance ranks obtained from this study are consistent with the findings of Yamane et al. (1996) and Liberg (1983). The dominance rank assigned by the staff members of the Feline unit was mirrored by the weight of each cat, Sunny being the heaviest, followed by Brock and Titan, and Bransen. In this case age does not strictly match the assigned dominance rank. Sunny is the oldest at seven years of age and the heaviest, but Bransen is five years old, two years older than Brock and Titan yet he is both the lightest and the most sub-ordinate male in the group.

Despite the lack of any significant differences between the three sub-ordinate males Brock, Titan and Bransen, the general trend in the female response does appear to follow the dominance ranking. Sunny, the most dominant male, elicited the greatest level of interest from the females. Bransen, the most sub-ordinate of all the males tended to elicit the least interest overall, being significantly different to Sunny for all parameters except latency. Some of this lack of female interest was ameliorated once Bransen's high level of shared relatedness with many of the females was taken into account.

Brock challenged Sunny frequently, resulting in fights and injuries. Titan retreated from threats from both Sunny and Brock suggesting that Brock held a higher ranking than Titan did. Feline unit staff also assigned Brock a ranking below the dominant male Sunny, and above Titan. The female response to these two males, Brock and Titan, was ambiguous. Although they were on the whole preferred more than Bransen and less than Sunny but these differences were not necessarily significant. The female preference for Brock and Titan alternated with the parameter measured. Occasionally there was a significant difference between Sunny and the two intermediate males, Brock and Titan, but sometimes there was no difference.

Sunny clearly had the greatest appeal to the females, even when the effect of relatedness was not accounted for. What is it that makes Sunny so attractive? The most obvious attribute that Sunny possesses is of being a dominant male. He is the most dominant male out the four males kept for breeding at the Feline unit. Sunny is also in the age and weight ranges that is comparable with other males of dominant status (Yamane et al., 1996; Ishida et al., 2001).

Sunny is the oldest male amongst the group of entire males, and he is likely to be the male most well known by the females. Eight females in the study group had experience of Sunny as a mate, or potential mate, when the breeding male is put in the same pen with the breeding females. The other females in the study group may well have experienced Sunny, and the other males, when they were living in a pen next door to the males. The entire males certainly interact with neighbouring oestrus females when there is only wire fencing between them (per observation). One pen was situated adjacent to the three males Sunny, Titan and Bransen after they were separated from Brock. Although these differences in female experience could not be controlled there may have been some effect on female interest. Sunny may have gained popularity because simply because he was the most familiar male.

At the time of this study neither Brock nor Bransen had been allowed to mate. Titan had been used as breeding male for one season with a small group of females. Sunny was the most frequently used breeding male and had been so for several years. The mating history of the females was rather diverse with some females having mated with Sunny only, Sunny and Titan, Titan only, and in one case with the previous breeding male from the unit who is no longer alive. The effects of different mating histories could not be tested as numbers were too low and histories varied.

Kola and Tori showed a preference for Sunny despite sharing an additive relatedness of more than 0.5. These two were the only females with such a high level of shared relatedness with Sunny that still held a preference for him. These two females had been mated to Sunny although both females had failed litters. This could possibly indicate a female preference for males with whom she had previously mated.

In some species, young females will follow the mate choices of older, experienced females (Dugatkin, 1992; Clutton-Brock and McComb, 1993). In guppies, naïve females will choose to mate with the same male that they have observed other females mating with. This imitation of mate choice is strongest when the female observed choosing a mate is older and in good health (Dugatkin, 1992). The Feline unit is open plan and most of the cats living in the colony can potentially smell and see each other. Sunny was used as the main breeding male for several years. It may be that other females living in the unit have witnessed Sunny as a viable mating partner for other females and therefore show preferential interest in his scent.

3.46 Good Genes and Dominance

Dominance has frequently been cited as being the most desirable quality in a male (Cox and Le Boeuf, 1977; Clutton-Brock et al., 1982) and extrapolated from this assumption the premise that should females choose, they should choose dominant males (Natoli et al., 2000). Dominant males should in theory have good genes, being generally older males who have proven they can survive and are strong. Males that are strong can then fend off competitors and have a greater chance of monopolising mating opportunities (Say et al., 2001). Indeed most research on rural free-living domestic cats would indicate that being a dominant male is a successful reproductive strategy.

Feral rural-living male domestic cats will usually leave their natal territory in order to find unrelated females to mate with. This male dispersal ensures that inbreeding with close kin is avoided. Therefore in the rural environment, a resident male is less likely to be related to the females living in his territory. Discrimination against close kin mating by an oestrous female may hold little relevance in an environment where she is unlikely to be courted by a related male. The dominant male reproductive advantage may be the logical outcome of male-male competition, and female monopolisation by a dominant male rather than an indication of female choice for dominant males. Monopolisation of an oestrous female is a particularly effective reproductive technique for the male, but it does however have to balance against possible mating opportunities with other females in oestrus at the same time (Say et al., 2001). When there are fewer oestrous females available, a dominant male will monopolise an oestrous female and sire more kittens per litter than when there are other oestrous females available. Therefore in rural environments, or when group-living females cycle asynchronously, dominant males have a better reproductive rate than sub-ordinate males (Say et al., 1999) and dominance is a highly effective reproductive strategy.

However when females form large groups around an abundant food source (Macdonald, 1983; Macdonald et al., 1987), the advantages of dominance become obscured (Say et al., 2001). There is less pressure on kin males to disperse as both food and unrelated females are plentiful (Pontier and Natoli, 1999). In this situation the females' ability to discriminate against kin males is useful in the prevention of inbreeding. Synchronous oestrus cycles and higher numbers of courting males prevent female monopolisation by one dominant male and female choice becomes apparent (Ishida et al., 2001).

Potential physiological and temperamental correlates with dominance may be the indirect methods by which females can assess the quality of a male. Dominance rank has been found to correlate with various physiological states. Dominant male baboons produce higher levels of insulin-like growth factor (IGF) I, a growth promoting and anabolic hormone, furthermore the differing levels of IGF-I are thought to be a consequence, rather than a cause of the social rank held by the male (Sapolsky and Spencer, 1997). Metabolic rate, cortisol levels and testosterone have also been correlated with dominance in various species (Fox et al., 1997; Cutts et al., 2001). Behavioural style can be used as a predictor of dominance in primates (Morgan et al., 2000). Behavioural styles associated with social rank have also been found to contribute to the endocrine correlates of dominance rank (Virgin and Sapolsky, 1997).

In the domestic cat, felinine is a urinary constituent that is thought to be a precursor for a pheromone to attract female mates (Hendriks et al., 1995c). Felinine is testosterone dependent and is biosynthesized from cysteine and possibly taurine (Hendriks et al., 1995c). Entire males have high felinine excretion rates and this impacts on the sulphur-containing amino acids that a male requires, thereby reflecting how successful a male is at acquiring high quality food. The pungency of male's urine, which is caused by the amount of felinine the male excretes may be an honest signal by which a male can advertise his 'fitness' to potential mates and competitors by urine marking (Bradshaw and Cameron-Beaumont, 2000; Hendriks et al., 1995c).

3.47 Do females choose?

There is a growing body of evidence showing that females can recognise close kin (Liberg, 1983; Yamane, 1998; Ishida et al., 2001) which is supported by the results obtained in this study. Ishida et al. (2001) observed that oestrous females would not co-operate with kin males during courting, mounting and attempting coitus. Females conceived more kittens from visiting males per copulation than they did for group males (Yamane, 1998). Visiting males are more likely to be unrelated and dominant, as only the more dominant males will visit other groups in search of mating opportunities, indicating that there may be some preference for dominance in a potential mate. However the dominance rank of visiting males can be misleading as visiting males often hold ‘courtship ranks’ which are lower than their home territory dominance rank, and do not reflect the male’s quality.

Natoli et al. (2000) observed that the males accepted by females for mating belonged to different classes of age, weight and dominance rank as assigned by the outcome of agonistic interactions between the males. They concluded that the mating pattern appeared to be a situation where “male cats mate randomly, exploiting a suitable moment, and females do not exert mate choice” (Natoli et al., 2000). However six of the fifteen females observed being courted were found to accept certain males for mating significantly more frequently than other courting males, but these “preferred” males were different individuals for each female (Natoli et al., 2000).

Idiosyncratic mate choice by different females may be a selection for genetic compatibility (Penn, 2002). Many species have been found to prefer mates with a different major histocompatibility complex (Ober et al., 1997; Olsen et al., 1998; Penn and Potts, 1998b). This can act as a function of inbreeding avoidance, but it can also select for a genome wide compatibility between mates that will confer greater viability on their offspring (Penn, 2002). Aspects of an individual’s history, such as prior mating experience or temperament may also influence mating choices. Females may also choose for good genes, and males that are healthy or carrying no parasites (Penn, 2002). All of these factors may potentially influence female choice, interacting together. Further research is needed to determine the importance of dominance and relatedness, past experience and temperament on potential female choice in the domestic cat and whether preferences for the urine of certain males correlates to mate choice or paternity.

Leyhausen (1979) asserted that dominance was irrelevant when courting a female, as “choice of partner is something that is almost always decided by the female”, citing examples of free-ranging and captive female domestic cats who remained “faithful” to low-ranking males for years with little interference from competing dominant males.

3.48 Further Research

As yet no studies have investigated the effects of male dominance on female mate choice in the domestic cat under controlled conditions. In order to determine the exclusive effects of dominance on female choice several criteria need to be addressed. Females should be tested using males that are unrelated and unfamiliar with a compatible major histocompatibility complex (should this be a relevant feature for domestic cats), in excellent health and kept in standard conditions. Several possibilities arise.

Several separate groups of males should be formed and allowed to establish a stable hierarchy. Once tested for female preference, groups can be mixed and a new hierarchy allowed to re-establish, so that once dominant males may now be in a group where it is now the most sub-ordinate. Similarly the most subordinate male from another group may now be established as the most dominant male in the new group. In this manner any possible effects that are bestowed on the animal as a result of dominance can be determined. Changes in the physiology of animals as a result of their social rank, rather than as the cause, have been observed in primates (Sapolsky and Spencer, 1997).

Alternatively, dominance may be the result of the intrinsic quality of the male (Morgan et al., 2000; Fox et al., 1997; Cutts et al., 2001). In this case, females could be given the choice of several unrelated males that have been established as being consistently high-ranking dominant males to determine whether any female choice is apparent when all the males are of a similarly high dominance rank.

Even if females do actively select the paternity of their offspring, such a mechanism would not eliminate the importance of male-male competition as part of the male reproductive strategy (Birkhead and Moller, 1993). Furthermore, this study has no replication of dominance rank. Although Sunny was indisputably the most dominant male amongst the four males kept at the feline unit, there was no other comparable, indisputably dominant male to compare the female response with. Indeed there is no other comparable male for any of the hierarchical rankings in this study. Bransen may not be of great interest to the females, not because he is the most sub-ordinate of the males, but for some other reason unique to him as an individual, such as his highly homozygous genome. Indeed all the female responses to each of the four males presented may be no more than an individual male effect without any repercussions on the social dominance rank of each male.

3.49 Parameters

Sniff duration and the number of visits paid to each sample appear to be useful parameters to record as description of each female's behaviour. Sniff duration and visits showed significant differences in the responses shown to the urine samples that the females were exposed to. Flehmen was more difficult to observe, as the body language appears quite similar to the position that cats adopt when they are sniffing. Flehmen is also sometimes performed with the head pointing down and it can be difficult to see. In this study the camera angle that allowed full view of the observation room compromised the view of the cats that allowed a close up view of the cats should they perform flehmen. However this compromise allowed better observation of the cats' behaviour when they were moving around the room. Flehmen could provide an accurate measure of each cat's interest in the urine samples should it be able to be clearly observed. The low level of flehmen recorded in this study is certainly a result of many performances of flehmen not being observed. The number of flehmen recorded for oestrous females is lower than the number of flehmen recorded for the females in anoestrus (see chapter two). This will be due to the changes in camera angle rather than an accurate record of the number of flehmen responses shown by the cats as females domestic cats are known to perform flehmen more frequently in oestrus than in anoestrus (Bradshaw, 1992).

Latency was the only parameter that did not appear to reflect any of the effects shown in the female response. No significant difference was shown between any of the males, there was no relatedness effect and no interaction between the two. Furthermore there was no consistency shown in the latency to approach the urine samples by each female as predicted by their responses towards the unrelated males. The only significant result was the female effect, indicating that there are differences between the females in their latency to approach the urine samples. Latency may be a more accurate reflection of a female's curiosity, or response to being placed in an unfamiliar room, rather than a good measure of preference. Although a female may be able to smell a urine sample from nearby without directly approaching and placing their noses directly above the urine sample, this is how all the females responded to the olfactory stimulus. Latency may not reflect a preference because the urine must first be approached before it can be sniffed or revisited. The interest a sample may hold cannot be determined before it has been smelt, therefore one may expect the approach towards visually identical patches to be random, until the differences between the samples have been determined by the cat.

3.410 Environmental Enrichment Potential

Chemocommunication in the Felidae is highly developed. In many species the female's sense of smell becomes keener during oestrus (Bradshaw, 1992) and males will increase their rate of urine spraying (Freeman, 1983; Natoli and De Vito, 1991). Conspecific urine holds a considerable interest for all felids (Gorman and Trowbridge, 1989) but what information it conveys is still largely a matter for speculation. There is some indication here that domestic cats may be able to determine kinship from the smell of a male's urine. Further research will be needed to determine whether such a preference can be used to accurately predict mate choice.

Captive breeding programs occasionally have problems with pair matching (Mellen et al., 1998) and occasionally, attempted pairings have resulted in mortal attacks on the female (Leyhausen, 1979; Lindburg and Fitch-Snyder, 1994). In solitary species, mating is an extraordinary moment in which the normal urge to repulse a conspecific must be overcome for copulation to ensue, and often the line between sexual and aggressive motivation can be quite slim (Lindburg and Fitch-Snyder, 1994). Appropriate information exchange may be able to go some way towards ameliorating the aggressive tendencies between mating pairs as well as contributing in other ways towards a successful outcome.

Placing the urine of potential mates inside the enclosure and observing the females' response may help in determining which male will be the best mate to introduce to her. Freeman (1983) observed that both the males and females of reproductively successful pairs of captive snow leopards (*Panthera unica*) expressed flehmen more than unsuccessful pairs.

Male cheetah allowed an opportunity to smell the enclosure of an oestrous female before the mating pair are physically introduced, responded to the female in a more solicitous manner rather than behaving aggressively or indifferently as they had previously (Lindburg et al., 1993). Employment of a protocol that allows for chemosensory investigation and a period of arousal can improve mating success (Lindburg and Fitch-Snyder, 1994).

Apart from this reproductive advantage, novel conspecific smells that are placed inside the enclosure may be an interesting environmental enrichment technique for felids kept in a restrictive environment that are still motivated to search for a mate. The events leading up to mating are an important part of the reproductive process and necessary for successful conception (Lindburg and Fitch-Snyder, 1994). Reproduction is still extremely relevant to animals that are not involved in captive breeding programs and this aspect of felid biology is likely to provide an abundance of environmental enrichment possibilities.

The extent of sexual attraction and mate choice in felids is not well understood. The results from this study infer that female domestic cats may discriminate against close kin when they are in oestrus. Any kind of preference suggests that suspicions of mate choice may be warranted. In captive breeding programs, reproductive failure often leads to suspicions that one member of the pair is infertile, when the problem may be incompatibility with the only mate available (Lindburg and Fitch-Snyder, 1994).

Chapter Four

Environmental Enrichment for Captive Felids Using Olfactory Stimulation

Sam and Roly sunbathing

"The Cheshire Cat only grinned when it saw Alice. It looked good-natured, she thought: still it had very long claws and a great many teeth, so she felt it ought to be treated with respect."

Lewis Carroll

Abstract

Six cheetah, two serval and a tiger were presented with various scents including synthetic feline facial pheromones, male domestic cat urine, mouse faeces, peppermint essence and catnip. Animals were housed at Orana Wildlife Park, Christchurch, New Zealand. The number of sniffs and the number of visits each cat paid to the various scents were recorded as an indication of each animal's interest in that odour. The overall response to the scents was low, although all the scents elicited a response significantly different to the control 'water' scent. The female cheetah and serval in this study responded to the synthetic feline facial pheromones by sniffing it significantly more than the other scents and by rubbing against the scent source, however the males did not show this response. The potential of scent as a tool for environmental enrichment of captive felids needs further investigation.

4.1 Introduction

Environmental enrichment seeks to stimulate the senses, by modifying the animals' external world in order to improve the quality of psychological and physical wellbeing (Newberry, 1995; Shepherdson, 1998). Several considerations are consistently applicable when designing an enrichment scheme. Animals benefit from a measure of control over the environment in which they live. Allowing animals to develop behavioural responses that have a functional outcome, such as being able to withdraw from stressful situations, can improve welfare (Wemelsfelder, 1984; Carlstead et al., 1993a).

Increasing the complexity of the captive environment can provide animals with more opportunities, supply novel experiences and induce a wider range of behaviours. Increasing the range of behaviours is commonly considered to represent effective enrichment (Chamove, 1989). Whatever methods are chosen in an effort to enhance the lives of captive animals, they must be appropriate to the sensory world of the target species (Poole, 1998). Social interaction, where it is appropriate to the species, food presentation methods, and novel features inside the enclosure are but some of the methods used to enrich the environment of captive felids (Mellen et al., 1998). Novel objects and odours are used to induce a greater variety of behaviours and increase activity. Such novel materials inside the enclosure can elicit elements of hunting behaviour, investigation, play and social interaction (Mellen et al., 1998).

Sight, hearing, taste, touch and smell are five of the senses by which the external world is perceived. The order of importance of these five senses will vary with the environment and niche each species occupies. Carnivores, in general, use smell to inform others of their territory boundaries, to find and attract mates, to improve social standing, and to announce their presence (Verberne and Leyhausen, 1976). Smell is an important and highly developed part of all felids' sensory perceptions.

Olfactory stimulation may be an effective means of enriching the environment for many animals. This is particularly true for amphibians and reptiles. The primary sensory modality for snakes and many other species of amphibians and reptiles is olfactory. Snakes, such as rattlesnakes will track prey by following their scent trail (Hayes et al., 1998). Western toads (*Bufo boreas*) can learn to identify prey insects by smell (Dole et al., 1981). Both prey and conspecifics can leave scent trails and reptiles and amphibians can track these scent trails quite precisely, sometimes over great distances. In captivity an animal's scent may be quickly distributed throughout an enclosure making it difficult for resident animals to locate prey and mates. This is referred to as olfactory saturation (Hayes et al., 1998). Conversely the routine cleaning of enclosures may deprive animals, more particularly mammals, of important olfactory information from urine, faeces, and sebaceous gland secretions. Male mice (*Mus musculus*) will significantly increase their rate of urine marking to compensate for the removal or replacement of marked objects inside their enclosure. Such increased urine marking causes a corresponding increase in the size of the coagulating gland and the testes (Collins et al., 2001)

Although olfactory stimulation is frequently recommended as a means of environmental enrichment (Forthman, 1998; Hayes et al., 1998; Mellen et al., 1998) few experiments have been conducted using scents as a technique to enhance the environment of captive animals. Powell (1995) evaluated the effects of various environmental enrichment techniques, including olfactory stimuli on African lions (*Panthera leo*). The scents tested were musk cologne, peppermint extract, allspice and almond. All these scents modified behaviour, specifically the lions' sniffing and flehmen behaviours, to some degree. Powell (1995) also reported using a commercially produced deer hunting lure made of extracts from the tarsal glands of the male deer during the rut. This increased not only sniffing and flehmen in the lions but also effected increased activity including rare behaviour such as social play and chasing.

Carlstead and Seidensticker (1991) used commercial hunting lures made of male and female bear urine to enrich the environment of an American black bear exhibiting stereotypic pacing. These scents significantly reduced pacing during the mate-seeking season. Pacing during the enrichment trial was significantly lower than during the same season the previous year without the olfactory stimulation.

4.11 The Relevance of Odour

Chemocommunication is a vital and integral part of all felid lives. The males and females of most felid species spray urine to mark territory and females spray to advertise oestrus (Bradshaw, 1992). Urine spraying is considered the primary mode of scent marking in many felids, including cheetah (Caro, 1994), tigers (Smith et al., 1989) and servals (Mellen, 1993) as it is both quick and effective, and leaves persistent olfactory cues (Feldman, 1994).

Cats will rub their head and face area on objects and on conspecifics. Sebaceous skin glands are well developed in this area (Verberne and De Boer, 1976). Scent marking via the spreading of facial secretions is likely to operate on a smaller scale than urine marking (Bradshaw, 1992). Rubbing generally differs between females and males and between older and younger cats, indicating that it may convey information regarding status (Feldman, 1994). Studies of feral domestic cats have revealed that rubbing by one sociable adult against another is common in well established groups, but is more likely to be performed by a subordinate individual towards a dominant one (Macdonald et al., 1987). This appears to be true for lions as well (Schaller, 1972). Rubbing behaviour may symbolize the peaceful intentions of the animal, indicating that it belongs to the group and has been accepted by it. Such acceptance is especially required from the powerful males. This may explain why lion females and cubs rub the males quite frequently, whereas the males seldom return the gesture (Schaller, 1972).

The cheeks and neck may also be rubbed against novel or odoriferous substances in the environment. Rubbing in this situation is also often associated with rolling, sniffing, licking and flehmen (Wemmer and Scow, 1977). There are two main differences between recumbent head rubbing and the scent marking variety. Recumbent head rubbing is in response to strong novel odours such as carrion, vomit and the faeces of unknown animals and catnip (Wemmer and Scow, 1977; Reiger, 1979). Head rubbing as scent marking is part of the daily routine of all felids (Wemmer and Scow, 1977).

Reiger (1979) divided the scent sources into five different groups: food, chemicals and perfumes, catnip, urine and faeces of other species, and scent markings of conspecifics or an individual's own scent. There is a strong relationship between scent rubbing and feeding. Although some scent molecules from the animal must be transferred onto the food, it is more likely that the odour of the food is transferred onto the animal (Reiger, 1979).

Reiger (1979) suggested that scent rubbing serves as an odour camouflage allowing the carnivore to cover its own body odour and thus increasing the chances of approaching potential prey animals without untimely detection. Fox (1971) proposed that rolling on an unfamiliar scent functioned to reduce novelty by combining the new scent with an individuals own body odour, the animal could then habituate to the novel odour as it wore it. Some carnivores scent rub on new and unfamiliar objects (Glickman and Sroges, 1966; Kleinman, 1966; Bogue and Ferrari, 1976), supporting the theory that scent rubbing might reduce novelty by depositing its own secretions.

Novel scents might be worn as a kind of 'perfume'. It is possible that a social animal, which returns scented with a novel odour, will experience greater social investigation by conspecifics than an "unscented" individual (Fox, 1971). In this situation rolling in a strong or novel odour may serve as an olfactory social attractant. This theory implies that social carnivores would scent rub more frequently than solitary carnivores. This assumption appears to be true for the phylogenetically oldest scent rubbed area (Reiger, 1979). The back is rubbed more frequently in social canids than by solitary canids, which generally prefer more cranial areas of their bodies for scent rubbing. This is also true for felids (Reiger, 1979). References about scent rubbing the back are found mainly for lions, the most social cat species (Schaller, 1972; Reiger, 1979). The fact that an individual cannot easily reach its own back further suggests that a primary function of scent rubbing is as an olfactory social attractant. Odour rubbed onto more cranial areas such as the head, neck or cheek would be more likely to be smelled by the animal itself. These are also the areas most often rubbed on species specific scent marks, indicating a possible reassurance function similar to that of some scent marks (Ewer, 1973).

4.12 Scent and Hunting

Cats have developed specialised hunting techniques that generally require concealment for success (Panaman, 1981; Bradshaw, 1992). There is plenty of evidence to suggest that all species of cats are opportunistic hunters (Schaller, 1972; Turner and Meister, 1988; Caro, 1994), taking prey randomly as they are encountered. Most species of cat will also scavenge if the opportunity presents itself (Schaller, 1972; Turner and Meister, 1988).

Domestic cats (*Felis catus*) have also shown searches for prey that are not arbitrary. Cats have been seen 'methodically' entering and examining rabbit burrows on Maquarie Island (Jones, 1977), and Leyhausen (1979) described observing cats which returned to the exact location of an earlier successful capture, days or weeks after the first event.
Domestic cats that are experienced hunters have reportedly followed urine stained mouse trails to the entrance of the mouse burrow (Turner and Meister, 1988). Schaller (1972) observed lionesses sniffing out a gazelle they had been hunting which had disappeared into high grass. Lions have also used their sense of smell to scavenge kills from other predators (Schaller, 1972).

4.13 Scents

Catnip

The herb catnip (*Nepeta cataria*) is commonly used as a stimulant for domestic cats. The active component in catnip is the compound nepetalactone, which elicits an intense reaction from some individuals, but this behavioural significance is still unclear (Bradshaw, 1992). There is no single behaviour that is considered the standard response to catnip. Cats react with sniffing, licking, a combination of face rubbing and body rolling, and often play which may consist of behaviours such as batting, biting and pouncing. This response is shown in both males and females, whether they are neutered, intact or even pregnant (Hill et al., 1976; Bradshaw, 1992).

No adaptive consequence has ever been shown for the catnip response (Palen and Goddard, 1966) and it may be that the reaction to nepetalactone, and similar compounds found in other plants may just be an evolutionary coincidence (Hill et al., 1976). The catnip response is genetically inherited in domestic cats as a dominant autosomal gene, therefore not all domestic cats will exhibit the characteristic catnip response (Bradshaw, 1992).

Other felids show a similar response toward catnip. Hill et al. (1976) tested the effect of catnip on six different species of wild felids, bobcats (*Lynx rufus*), lions (*Panthera leo*), jaguars (*Panthera onca*), cougars (*Panthera concolor*), leopards (*Panthera pardus*) and tigers (*Panthera tigris*). Lions and jaguars responded to catnip consistently. Leopards were deemed partial responders as they spent less time reacting to the catnip than did the lions and jaguars. Bobcats, cougars and tigers did not respond (Todd, 1962; Hill et al., 1976).

The response to catnip in lions and jaguars continued indefinitely, up to 60 minutes, and there did not appear to be any observable decline in interest over time (Hill et al., 1976). Conversely domestic cats exhibit responses that seldom last longer than fifteen minutes and are followed by a one hour long refractory period (Todd, 1962). Adaptation to the presence of catnip in the environment may explain the limited response, and refractory period shown by domestic cats (Hill et al., 1976).

Peppermint

Peppermint (*Mentha piperita*) is best known as a remedy to stomach problems in humans. It has carminative, antispasmodic, choleretic, analgesic and antiviral properties (Spirling and Daniels, 2001). In aromatherapy, peppermint is used as both a stimulant and holistic relaxant (Compton, 2000) and it has been shown to have both sedative and stimulating effects on mice (Della Loggia et al., 1990; Umenzu et al., 2001).

Powell (1995) used peppermint extract as an olfactory environmental enrichment aid for captive lions due to its botanical relationship to catnip. It elicited the strongest reaction out of the scents used in that study causing a significant increase in sniffing and flehmen when placed in the enclosure. Of the scents used in that study, including musk cologne, allspice and almond extract, peppermint was found to elicit the strongest reaction

Mouse Faeces

Faeces of mice and rats will be used as the scent of a prey species in the enclosure and to induce elements of hunting behaviour in the captive animals. Lions respond ecstatically to zebra or rhinoceros faeces introduced into their enclosure, by rubbing and rolling in the dung (Marvin Warren, animal keeper, Orana Park, per communication). Verberne (1976) found that domestic cats responded to the urine of male rabbits with enthusiastic rubbing and rolling, and occasional flehmen.

Hiding food inside enclosures has been an effective method of environmental enrichment for many species of felid (Carlstead et al. 1991; Law, 1993; Shepherdson et al., 1993). Animals track the food caches by smelling out where they are hidden.

In a similar manner the faeces of potential prey could indicate a hunting opportunity and should elicit interest from the cats. The faeces of mice and rats were chosen as they should be interesting to the smaller felids, such as the serval, and still provide novelty to the larger felids.

Urine

The urine of conspecifics can convey a wealth of information. Urine is used to mark territories (Verberne and Leyhausen, 1976; Bradshaw, 1992), intimidate competitors (Caro, 1994), and advertise oestrus (Verberne and De Boer, 1976). Both domestic cats and undomesticated felids communicate using olfactory 'signposts' of urine deposited in conspicuous places (Wemmer and Scow, 1977).

The urine of all mammals shares some constituents of hormones or metabolites of hormones (Verberne, 1976), and may therefore hold elements of interest for other species. It is believed that urine also holds species specific messages, including components that allow the 'receiver' sniffing the urine to identify the species of the animals that deposited the urine (Brahmachary and Dutta, 1981). If this is so, then animals will be able to distinguish the olfactory marks of other species from those of conspecifics. Urine of an entire male domestic cat could indicate the presence of an unknown predator inside the felid's territory. Although interest in these markings is primarily shown by conspecifics, urine of competing predators may also elicit interest in an environment that is otherwise devoid of competitors.

Interspecies competition by predators that share resources can lead to klepto-parasitism and the killing of cubs or even adults by larger predators. There are many accounts of situations where carnivores have been killed by their competitors (Polis et al., 1989; Bailey, 1994; Caro, 1994). Lions will frequently kill smaller carnivores, such as cheetah or serval, if an opportunity is presented (Caro, 1994). Young cheetah have been observed chasing serval or jackals, although this has usually been in the context of practicing their hunting skill rather than a serious intent to harm these smaller carnivores (Caro, 1994). Whether it is in the context of a potential predator, or a potential prey, signs of a competitor in the vicinity may be of interest to felids.

Facial Pheromones

Pheromones are a part of most animals' intra-species communication repertoire. Pheromones released by one individual can affect and alter the behaviour of other individuals of the same species, sometimes this may also include the behaviour of the animal that released the pheromone (McClintock, 1998; Allaby, 1999). Feline facial secretions have been found to have a pheromonal content (Verberne and De Boer, 1976; Pageat, 1996). Facial rubbing in domestic cats is a behaviour seen in both sexes, typically beginning from the side of its chin to the base of the ear, sometimes developing into a roll (Verberne and Leyhausen, 1976).

A strong relationship was found between urine spraying and facial pheromones. Cats are unlikely to urine mark on objects that have been previously rubbed with facial pheromones (Ogata and Takeuchi, 2001). This connection led to the commercial production of Feliway (Sanofi Sante Nutrition Animale, France), a synthetic analogue of the feline facial pheromone. Feliway has been targeted for the treatment of domestic cats with unwanted urine marking problems.

Pageat (1997) found that feline facial pheromone (or its synthetic analogue, Feliway) appears to calm domestic cats when they are in stressful or unfamiliar situations. This relaxing effect has been put to use in veterinary hospitals, where cats treated with a synthetic analogue of feline facial pheromones have demonstrated earlier exploration of their cages, accepted food earlier, and groomed more than cats which were not treated with the pheromone (Griffith et al., 2000). It appears to be this calming function that is responsible for discouraging urine marking in domestic cats (Pageat, 1997; Frank et al., 1999; Hunthausen, 2000; Mills and White, 2000).

It likely that natural feline facial pheromones serve an orientation function (Ewer, 1973). They are deposited on boundaries of passageways and territorial zones (Leyhausen, 1979; Bradshaw, 1992). To the individual it seems to be a note to itself that "I have been here before", as opposed to urine marking which is thought to serve primarily as a message to others (Gorman and Trowbridge, 1989). Facial pheromones may also serve a social function. Head and face rubbing is a common greeting behaviour in all felids (Schaller, 1972; Wemmer and Scow, 1977; Macdonald et al. 1987). Apart from the visual and tactile aspects of rubbing against another individual, this behaviour helps deposit secretions onto the rubbed individual and vice versa (Reiger, 1979). It has been suggested that this may facilitate the creation of a group smell, allowing trespassers to be easily distinguished (Kerby and Macdonald, 1988). In the same manner that these pheromones function in geographical orientation, they can function in social orientation. Facial pheromones leave a mark telling the individual " I have been here before" and that the newcomer is a known individual and not an intruder (Turner and Meister, 1988).

If the facial pheromone is used in the context of "I have been here before" there is an implied message of familiarity and safety. Reassured that it is safe a cat may be more likely to relax. In terms of the Feliway solution, a neutral message that it is safe to relax could hold considerable appeal.

There are no published accounts of Feliway or feline facial pheromones being used for species of feline other than domestic cats. Rubbing behaviour is commonly reported for many species of felines (Schaller, 1972; Verberne and Leyhausen, 1976; Caro, 1994). Sebaceous glands have been identified around the head and neck area of lions (Schaller, 1972; Verberne and Leyhausen, 1976). Although the synthetic feline facial pheromone Feliway is based on the pheromones of the domestic cat, these pheromones may also be of interest to other members of the Felidae.

4.14 Aims

This study will investigate the possibilities of using olfactory stimulation as an environmental enrichment technique for wild felids held in captivity. Several scents that may be of interest to felids have been chosen. These include catnip (Hill et al., 1976; Bradshaw 1992), peppermint (Powell, 1995), the faeces of rats and mice (Reiger, 1979), domestic cat urine (Reiger, 1979; Bradshaw, 1992; Feldman, 1993), and Feliway (Sanofi Sante Nutrition Animale, France), a synthetic analogue of feline facial pheromones (Verberne and De Boer, 1976; Pageat, 1996). Each felid's response to the presentation of a novel scent will be recorded. The responses to each odour will be compared to determine what, if any, scent is of interest to the animals.

4.2 Materials and Methods

The use of scent as a tool for environmental enrichment for wild felids held in captive situations was investigated at Orana Wildlife Park between July and December, 1999. Six cheetah, two servals and a tiger were exposed to various scents: synthetic feline facial pheromone; male domestic cat urine; mouse odour; peppermint and catnip, in order to determine whether scent as an environmental enrichment can effectively modify felid behaviour.

4.21 Location

Orana Wildlife Park is New Zealand's largest wildlife park. It is situated on McLeans Road near Christchurch. The Orana Wildlife Trust is a registered charity that manages the park. The park aims to provide recreation and education on current conservation issues. The park also participates in international captive breeding programs for endangered species, including kiwi, oryx and cheetah.

4.22 Animals

Table 4.1 The species, sex, age and names of the cats used in the trial by pen group.

Species	Sex	Born	Name
cheetah	male	20/12/93	Mbili
cheetah	male	20/12/93	Tatu
cheetah	male	20/12/93	Moja
cheetah	male	6/11/97	Kaitaia
cheetah cheetah	female female	6/11/97 6/11/97	Kazkazi Yatima
serval serval	male female	13/02/93 13/02/93	Zulu Sheba
tiger	male	18/11/81	Sam
lion lion	3 males 3 females	20/08/83 17/09/87	Jomo, Tarma, Cemba Tombi, Tess, Kushka
lion	male	12/02/98	Toby

All of the animals used in this trial were housed at Orana Park (Table 4.1).

Cheetah (*Acyninox jubatus*)

Six cheetah, three adult males, and three sub-adult littermates consisting of two females, and one male were used. The adult males were six-year-old littermates. The sub-adult cheetahs were born in November 1997 and were nearly two years old at the time the observations were taken.

The three adult males were kept in separate but adjacent enclosures. The two sub-adult females were housed together and the sub-adult male was kept in the enclosure next to them. There are six separate cheetah enclosures and an exercise area at Orana Park. Three enclosures are on exhibit and three are off exhibit. The males' responses to scent patches were recorded while they were in the exhibit enclosures. The cheetahs are rotated through the various enclosures (on and off exhibit) on an irregular basis as a method of environmental enrichment. The exercise area is a paddock about 0.4 ha. in size. It is a large open area with long grass and there are several trees and bushes along the stream that runs along one side of the area. The exercise area is next to the enclosures on display. The exhibit enclosures are approximately 10 meters by 15 meters. Every day, the males were shifted one enclosure along in a constant rotation within the exhibit enclosures. The cheetah in the end enclosure next to the exercise area had open access to this paddock. All of the cheetahs have access to the exercise area when they are in the exhibit enclosures allowing them space to roam. Rabbits and birds such as magpies and hawks that come into the exercise area are treated as prey by the cheetah.

The sub-adult littermates were held off-exhibit during their sampling. There are three off-exhibit enclosures all of similar size, about 30m by 30m. These enclosures have scattered bushes and mature trees with open areas covered in long grass. All enclosures have a kennel the cheetah can shelter in. It has a bed of straw or shredded paper for warmth and comfort.

Tiger (*Panthera tigris*)

The Siberian-Bengal hybrid tigers, Sam and Rolly were 18-year-old male littermates born at Orana Park in 1981. Sadly Rolly had to be put down a month after the beginning of the project due to severe arthritis. All the observations of Sam were taken after Rolly died. The tiger enclosure is constantly on exhibit and is situated adjacent to the lion house. It is approximately 30m by 20m. Part of the enclosure can be closed off, but it is usually left open. The enclosure has an artificial pond, long grass, several small plants, bushes and two raised wooden platforms. There are also several large posts within the enclosure. These hold up the wire fencing "roof" of the enclosure and were used by the tiger as scratching and marking posts. Each night the tiger was put in a night cage and let out again in the morning. Sam was allowed access to the night cage on days when the weather was bad, as there is little other cover from the elements inside the enclosure.

Servals (*Felis serval*)

The servals were two 6-year-old siblings, one female, Sheba and one male, Zulu. Orana Park took in the servals in 1995, when the New Brighton Zoo closed down. They were captive bred in New Zealand. The serval enclosure is situated near the main park entrance, in a complex which also holds the meerkat and porcupine exhibits. The serval enclosure was a roughly circular shape, approximately 10 meters in diameter. The outer walls are made of concrete, made to look like natural rock. There was a two-meter fenced opening on one side. Adjacent to this there was also another viewing area with one-way glass. The enclosure floor was covered in long grass and tussocks. There are several raised platforms, with tree branches connecting them together. A heated indoor area was attached to the exhibit area, and the servals were kept inside every night and let out again in the morning.

Lions (*Panthera leo*)

The three adult males and three adult females were kept together in a non-breeding pride. One male was vasectomised, and the other two were castrated. At night the lions were kept inside the lion-house. During the day they were released into one of two adjacent enclosures. The smaller enclosure was approximately 20m by 10m. The larger enclosure was a field approximately 0.4 ha. in size. To gain entrance to the large enclosure the lions had to pass through the small enclosure. At the time this study began, a new juvenile male lion, Toby, arrived. Toby and the Orana pride alternated days inside each enclosure. The presentation of the scent patches to the lions was not included in this study. Due to the arrival of a new lion, all of the lions spent all day pacing up and down the adjoining section of fence between the two enclosures, roaring and charging at the new arrival. The new arrival spent most of his time in a similar manner. Initially the patch was presented, but it appeared to be completely unnoticed.

Diet

All the cats are fed a diet of red meat, alternated with whole or partial chicken or rabbit carcasses. The servals' diet is alternated with chick or mice carcasses. The larger cats also receive bones and whole or partial carcasses of sheep or goats to vary their diets. Anivite, a vitamin supplement, and calcium powders are dusted on the meat and vegetable oil is given to each cat with their food. Selenium is injected into each cat's meal once a month.

4.23 Scents

The six selected scents were considered to be of potential interest to felids. Scents were chosen based on possible biological relevance and prior use as an environmental enrichment tool (Powell 1995).

Each scent was applied to a 10cm square calico cotton patch, which was then attached to the fence using plastic clothes pegs. The patches with the pegs attached were washed without detergent and dried in a washing machine/drier before the scent was used. All the scents, which were in liquid form, were applied using a sterile plastic syringe prior to the presentation for the day. The cat urine and mouse faeces where placed in a pouch inside the patches each day before they were used. Once the patches were smelly they were kept individually inside airtight plastic bags until they were needed. Scents and patches were renewed after each presentation. During all stages of preparation the patches were handled using sterile plastic gloves to prevent any transference of scent from the observer.

“Feliway” (Sanofi Sante Nutrition Animale, France) -Feliway is a synthetic analogue of the domestic cat facial pheromone that is commercially produced.

Male Domestic Cat Urine -This was collected from an eight-year-old entire male Himalayan Persian kept for breeding at the ‘Cats Away’ cattery in Christchurch. Urine saturated sawdust was collected weekly to be put into the patches.

Rudducks Catnip Spray (Rudducks Laboratories Pty Ltd, Australia) -Rudducks Catnip spray is a commercial product of concentrated catnip.

Peppermint Essence (Hansells Ltd, New Zealand)

Mouse Faeces -Faeces were collected from the rats and mice kept at Orana Park. Initially gazelle faeces were used, but they did not appear to hold any more interest for the cheetah or the tiger than that of the mice. As the collection of the mouse faeces was unproblematic, compared to the gazelle faeces, which required zookeeper co-operation, the mouse smell was included in the study

Water from the tap was used as a control.

4.25 Catnip inside the Enclosure

The catnip extract was also sprayed onto posts inside the enclosures of the lions, the serval and the tiger, when they were still in their nighttime accommodations. As the cheetah stayed in the same enclosure both day and night, catnip extract could not be sprayed inside their enclosure. When the catnip extract was placed inside Sam’s enclosure, it was sprayed against two poles; one of these poles was frequently marked with urine, the other, much less frequently or not at all. The animals’ responses were observed as they re-entered their enclosure areas. In the case of the lions, the catnip was presented inside the enclosures, once to the old pride, and once to the new juvenile male while the old pride was still inside their nighttime enclosure.

4.24 Data Collection

There was a three-week habituation period, when just the cotton patches without scent were attached to the enclosures, to allow the animals to get used to both the presence of the patches and the presence of the observer. The experiment was conducted in the morning between 7am, when the gate was unlocked and 10.30 am when the park was open and visitors had begun to arrive. Observations were taken when the Orana Park staff were not present, as the keepers caused considerable distraction to the cats and to the observer. Each smell was presented four separate times to each animal throughout the duration of the project and the order of the cats and the presentation of the scents were randomised. Scent patches were attached to the cage fence in a standard position from which as much of the enclosure could be seen as possible. Once the patch was attached the observations began and continued for 15 minutes. Once the fifteen-minute observation period was complete the scent patch was removed. The behaviours (Table 1) were recorded using one-zero sampling every fifteen seconds (Altmann, 1974). The number of visits the cat paid the scent patch was also recorded. One visit consisted of the cat approaching the patch and sniffing it before walking away again.

4.25 Analysis

A split plot design (Systat Version 8) was used to analyze the responses to the scents presented. Each individual cat was represented as one whole ‘plot’ and each of the six scents was applied to each animal. Between ‘plot’ differences were represented by sex and by species. Because of the unequal combination of sex and species two models were used, one for the effect of sex and one for the effect of species type. The single tiger was excluded from the species analysis. The effect of sex and scent type was further analysed without Feliway and the control to determine any further gender differences between the response to the different smells.

Table 4.2 *Ethogram of recorded behaviours*

Visits
The animal approaches the patch, investigates and leaves again

Sniffing
Sniffing scent patches, air, enclosure features, or other objects

Flehmen
Mouth open, lips drawn back, face wrinkled, tongue out, following the sniffing of an object or cat (Mellen 1993)

Face rubbing
The cat rubs the side of its cheek from the tip of its nose along to its cheek, and may continue down its neck

Grooming
The cat licks itself or other cats, other cleaning behaviours

Rest
The cat is lying down or sitting with eyes closed and head down

Alert
The cat is lying down or sitting with eyes open and head up
Standing or walking

Headshake
The cat shakes its head usually seen following sniffing

Back rolls
The cat rolls onto its back

Spraying
Spraying urine on a vertical surface

Sharpening Claws
Claws of front paws are used to scratch surface

Vocalisation
Meow, hiss, spit, and growl

Pouncing
The cat briefly rears up on its hind legs before dropping both fore legs down on the object

Attack
The cat manipulates an object with its two front paws while upright, or with all four paws while lying down

4.3 Results

When the odour patches were presented each of the cats usually approached and sniffed the patch at least once. The most commonly observed behaviours were the cats either walking or sitting alert. If the scent patches were visited it often appeared to be as part of their routine pacing that took them past the area of fence that the patches had been attached to, often an animal would barely pause to sniff the patch before continuing.

Orana Park runs its own program of environmental enrichment, which was happening concurrently with the present study. Many of the environmental enrichment programs were unpredictable, often relying on whether the zookeepers had extra time in their busy daily schedule or the opportunistic presentation of enrichment possibilities that arose from the general running of the park. For example, on any given day at the park, the felids may be presented a large pile of rhinoceros faeces, or an entire carcass maybe placed inside the enclosure. Feeding times were also alternated on an irregular basis, so that the animals would not become complacent. The arrival of a keeper always attracted attention from the cats, as there was always the possibility of food. Occasionally the park enrichment schemes coincided with the presentation of the patch, and observations had to be postponed for another day. The presence of volunteers or keepers would also affect the behaviour of the cats resulting in delayed observations.

There were also 'natural' environmental enrichment opportunities. Various wild animals and birds that live free at Orana Park would have the misfortune of wandering into a felid's enclosure. The cheetah were frequently observed hunting rabbits, magpies and even hawks successfully, especially when they had access to the exercise paddock, which was large and had long grass and plenty of rabbits. The tiger was seen catching, plucking and eating a peacock that had wandered into his enclosure. Peacocks roam freely around the park.

The cheetah males were initially very reluctant to approach the patch at all. However, when the cheetah males were not being actively observed (observer attention had wandered) they chose these moments to approach the patch and investigated this novel addition to their environment. Particularly in the case of the adult cheetah males, a hide would have been useful. Unfortunately, due to the layout of the zoo, and time and budget constraints, and the regular housing rearrangement of the cheetahs within the enclosure, the use of hides was not feasible. However, the observer not looking directly at the males, while still looking out for any for interest a cheetah may show in the patch seemed to be the most successful compromise. While still allowing observation, the cheetah approached the patch more frequently than when previously directly observed.

Flehmen was not observed by any of the cats in response to the scent patches. One cheetah male was observed performing flehmen, after it had been moved into a new enclosure, but it was in response to urine on the ground that had presumably been deposited by the oestrous cheetah female that had occupied the enclosure the previous day.

Vocalisations were heard on one occasion from one of the adult cheetah males, however this behaviour was present before, during and after the patch presentation and appeared to be directed towards its male sibling that was out in the exercise area. The vocalisations stopped when its sibling returned to the adjacent enclosure.

Although other behaviours such as urine spraying and grooming were observed, their occurrence was so low that they could not be included in the analysis. The females responded to the Feliway patches by rubbing and occasionally licking them. This behaviour was only observed in the females, in response to the Feliway patches.

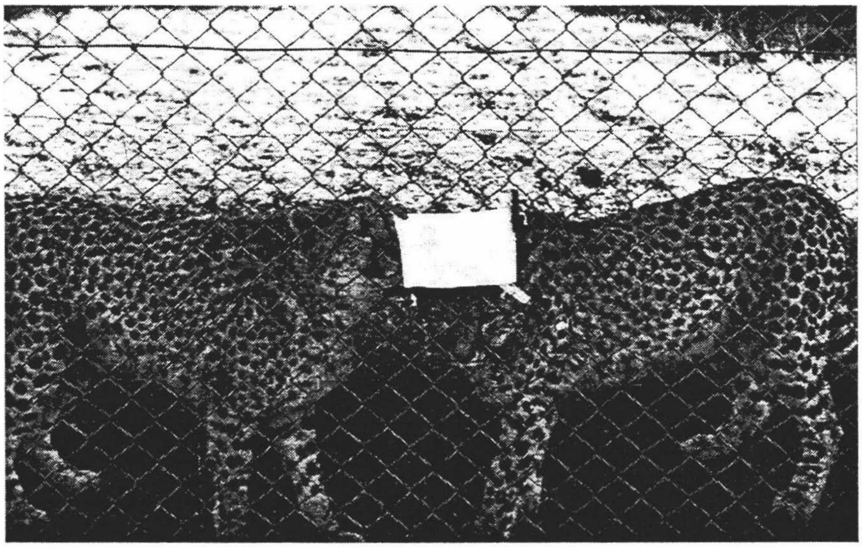
The number of sniffs and the number of visits to each scent patch were recorded as indications of each animal's interest in each odour.

4.31 The Effect of Odour and Gender on the Number of Sniffs

The overall effect on sniffing behaviour of exposure to all the smells (the smell effect) was significant (Table 4.3). The control of a blank patch sprayed with water was found to elicit a very low response averaging less than one sniff per presentation for each cat. The

Table 4.3. F-ratio and p-value for the effects of different smells, and the effects of sex (A) and species (B), for the average number of sniffs and visits.

Parameter	Smell Effect	Sex Effect	Species Effect	Interaction
Sniff (A)	F 5,35 =11.50 P <0.001	F 1,7 =6.57 P =0.037	- -	F 5,35 =3.63 P =0.009
Visit (A)	F 5,35 = 5.03 P =0.001	F 1,7 =4.84 P =0.064	- -	F 5,35 =0.46 P =0.801
Sniff (B)	F 5,30 = 6.86 P <0.001	- -	F 1,6 =1.93 P =0.214	F 5,30 =0.82 P =0.544
Visit (B)	F 5,30 = 9.08 P< 0.001	- -	F 1,6 =6.04 P =0.049	F 5,30 =5.79 P<0.001



Cheetah females, Yatima and Kazkazi investigate the Feliway patch male domestic cat urine, mouse, mint and catnip elicited a level of interest similar to each other, and Feliway elicited the strongest reaction (Figure 4.1).

Overall there was a significant difference in reactions between the sexes (Table 4.3) with females responding more to smells than the males. The sex by smell interaction was also significant (Table 4.3). Females sniffed more than the males, particularly in response to the Feliway patches (Figure 4.3).

The male cheetahs were generally more reticent about sniffing the patches than the female cheetahs. Feliway was of the greatest interest to all the cheetahs, particularly to the females. The Feliway also elicited the biggest response from the serval female. There was much less interest by the male serval and tiger in the Feliway patches (Figure 4.3).

The Feliway data was removed from the analysis in order to determine whether the significant differences in the smell effect and the sex effect were due to the response to Feliway. The sex effect ($F_{1,7} = 2.63$, $P = 0.149$) and the interaction between sex and scent type ($F_{4,28} = 0.29$, $P = 0.883$) were no longer significant when the Feliway data was not included in the analysis. Therefore the overall difference in response to the smells was largely due to the fact that all the females responded enthusiastically to Feliway, without the Feliway the sex difference in overall response disappeared.

However, even with the Feliway data removed, there was still a significant effect from the smells on all of the felids ($F_{4,28} = 7.59$, $P < 0.001$). This confirms that all of the smells used elicited a response from all of the felids that is significantly different from their response to the control patch that was sprayed with water.

The male domestic cat urine generated interest from all of the felines involved in this study (Figure 4.1 and 4.4). Apart from the interest, particularly from the females, in the Feliway, and the interest by the servals in the mouse scent (Figure 4.5, table 4.3), the male domestic cat urine was given the most attention overall. The tiger sniffed the urine

Figure 4.1 Overall mean sniffs from all animals in response to each scent.

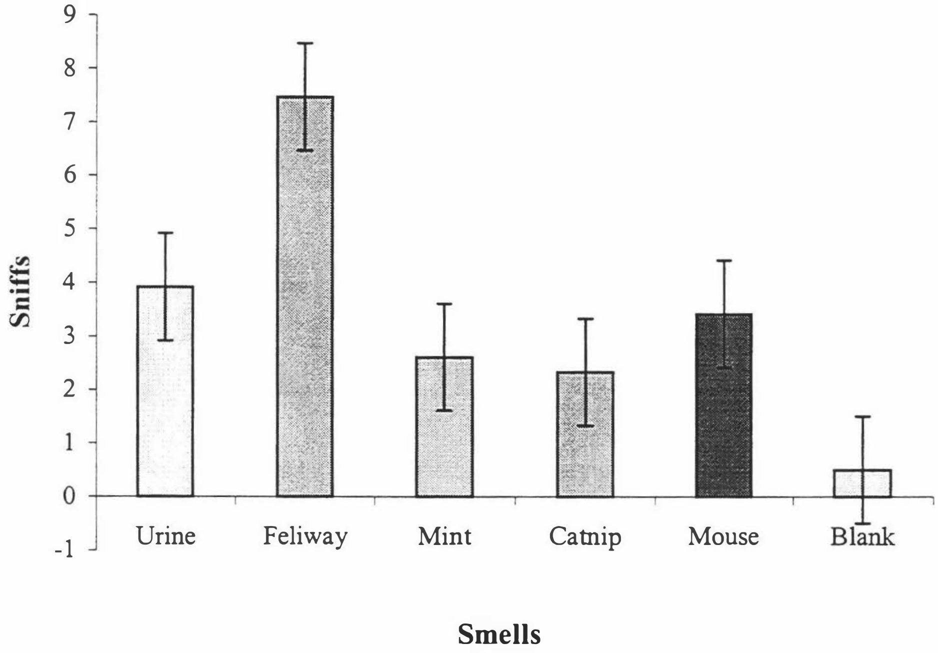


Figure 4.2 Overall mean number of visits from all the animals in response to each scent.

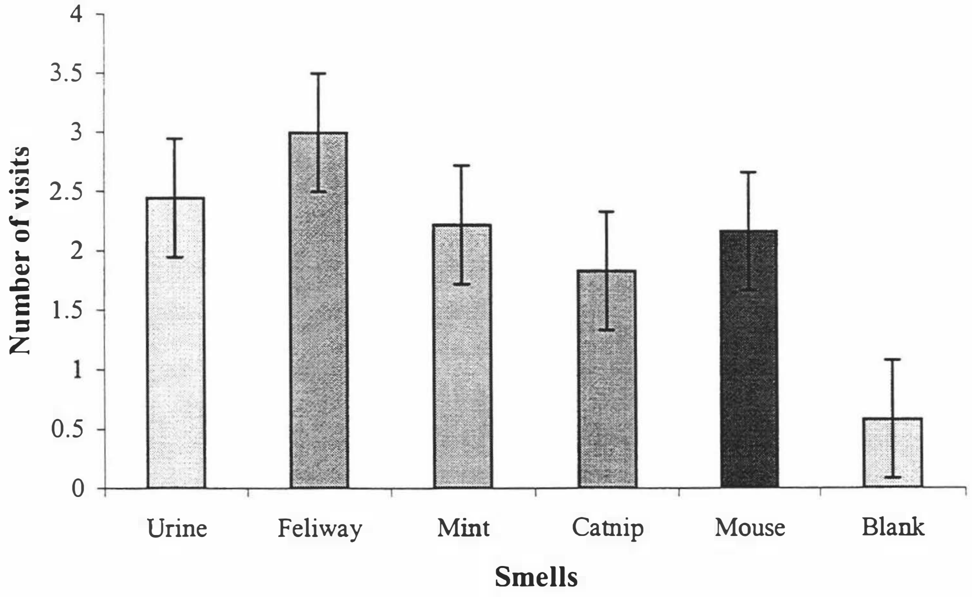


Figure 4.3 Mean sniffs and number of visits by each cheetah (C), serval (S) and tiger (T) to the Feliway scent.

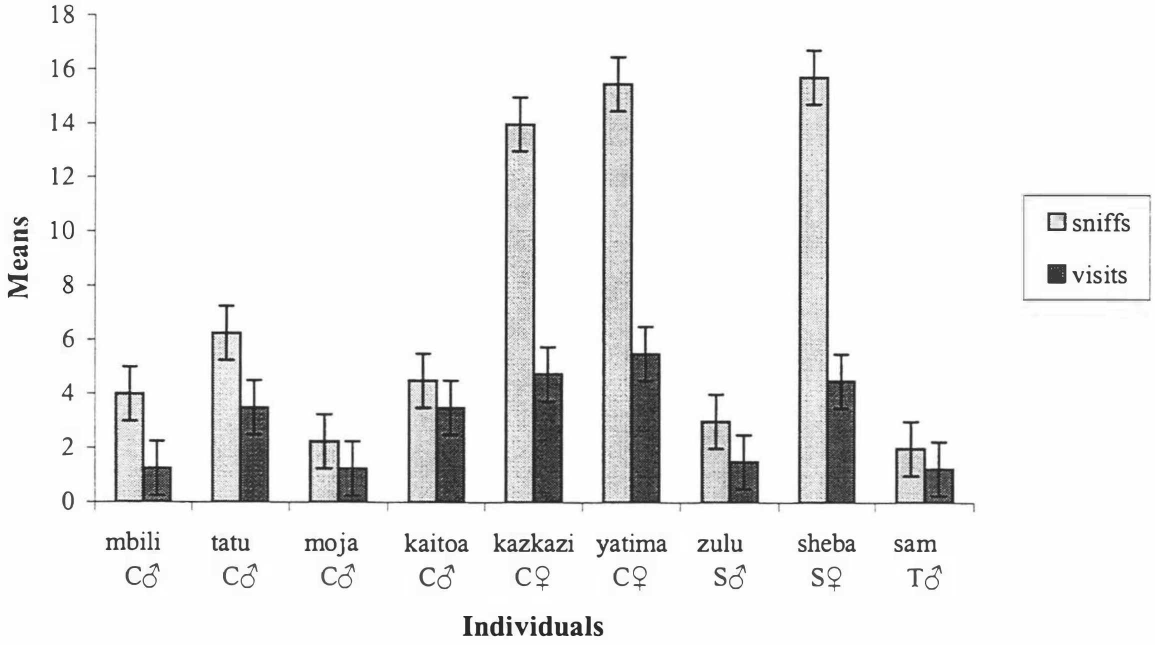
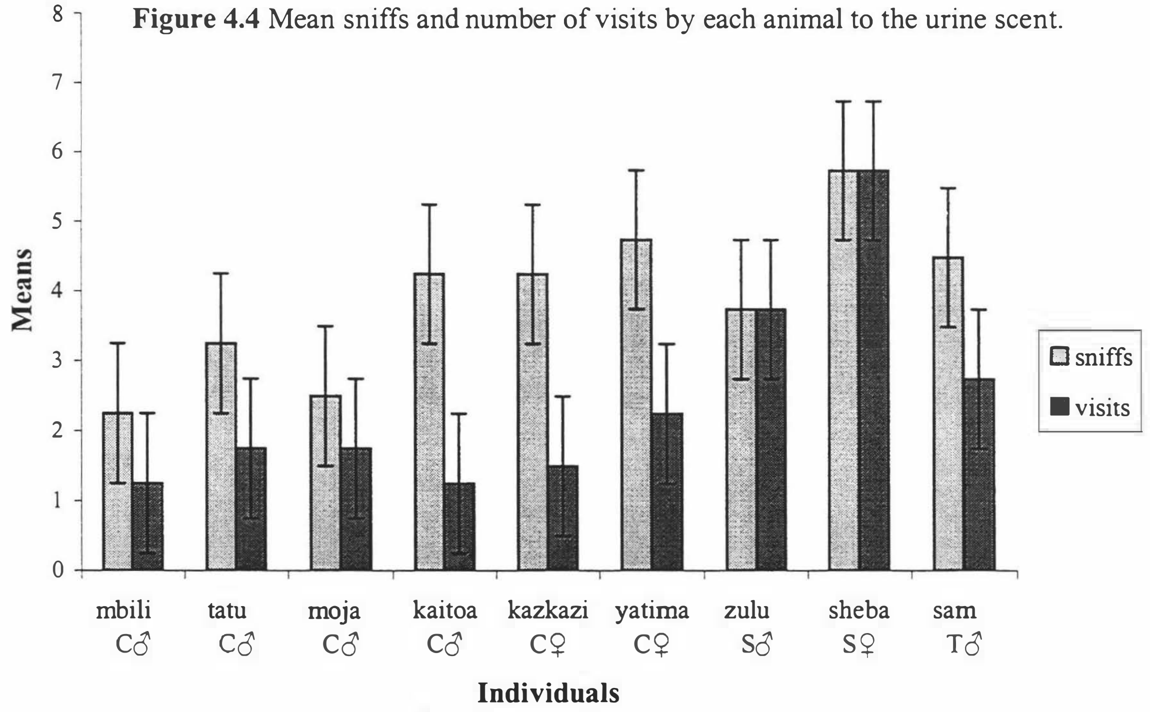


Figure 4.4 Mean sniffs and number of visits by each animal to the urine scent.



patches the most often out of all the smells that were presented to him. For the tiger and cheetah, the mouse scent was not of particular interest (Figure 4.5). The mint and catnip smells both stimulated a similar level of interest from all of the felids (Figure 4.1). When compared to the control, it can be seen that the mint and catnip were of some interest, though less so than the other scents.

The removal of both the Feliway and the water control from the analysis revealed the smell effect to be no longer significant ($F_{3,21} = 2.21$, $P = 0.117$). The remaining smells (urine, mouse, peppermint and catnip) elicited a similar level of response from all the felids. There was no significant sex effect ($F_{1,7} = 2.31$, $P = 0.173$) or interaction ($F_{3,21} = 0.11$, $P = 0.956$).

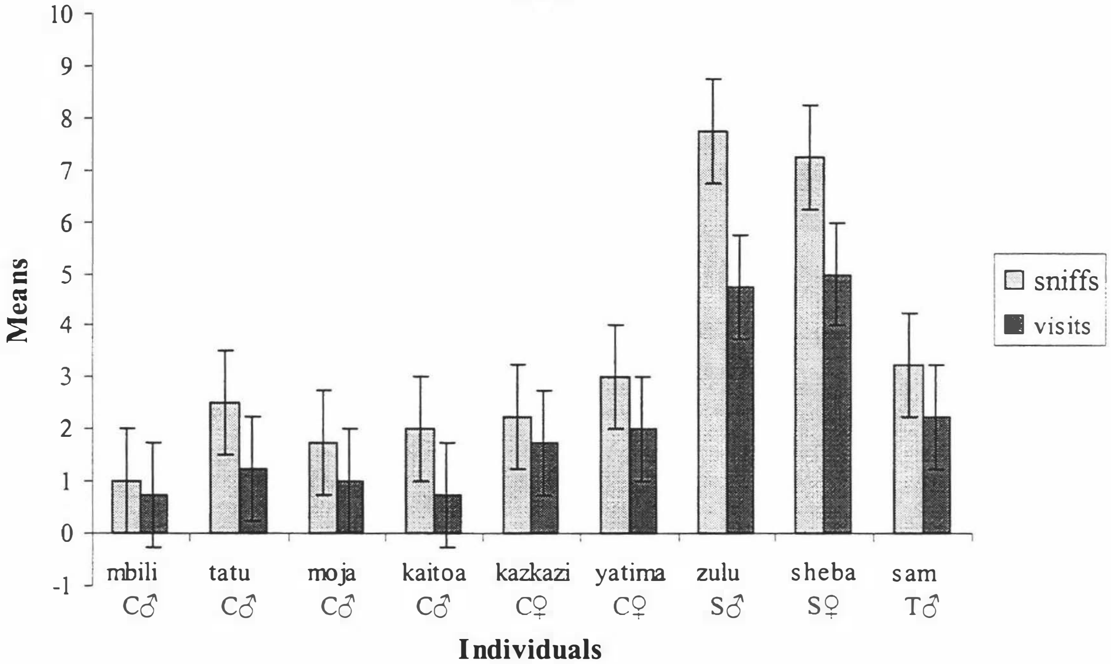
4.32 The Effect of Odour and Gender on the Number of Visits

The proportion of visits was similar to but less than the number of sniffs. Once again Feliway elicited the most interest but the difference is not as great as that shown by the data from the number of sniffs (Figure 4.2).

There was a smell effect on the animals as reflected by the number of visits they paid to each of the scents (Table 4.3). However, no significant sex effect and no interaction between sex and scent type were found when considering the number of visits to the patch for each of the cats (Table 4.3).

When the data from the felid responses to the water control were removed from the analysis, the smell effect became non-significant ($F_{4,28} = 1.32$, $P = 0.286$). This indicates that all the smells had an effect significantly greater than the control. However, there was very little difference between the number of visits elicited by catnip, mouse, mint, urine or Feliway. Feliway elicited a greater number of visits but this was not significant once analysed. If the number of visits is used as an indicator of the interest aroused by each smell, then all the smells appear to be of generally equal interest.

Figure 4.5 Mean sniffs and number of visits by all the animals to the mouse scent.



Female serval Sheba rubs against a Feliway patch

Overall the servals were the most frequent visitors to the scent patches, with the cheetahs visiting the least often, except for the Feliway patches. Feliway elicited the greatest number of visits from the cheetah, with the females visiting the Feliway patches more than the males (Figure 4.3). The mouse scent and domestic cat urine caused the greatest number of visits to the patch from both the male and female servals. The female serval returned to visit the scent patches the most out of all the individual felids, although this sex bias is not significant. The tiger paid the most visits to the catnip and domestic cat urine s, which are also the two scents that the tiger sniffed the most (Figures 4.6 and 4.7). The blank patches elicited the least visits from all the cats.

4.33 The Effect of Odour and Species on the Number of Sniffs and Visits

A smell effect was found for sniffs, and for visits (Table 4.3). Once again, all of the smells caused a greater response, in terms of sniffs and visits, than the control.

There was no significant species effect regarding the number of sniffs, however there was a small species effect when considering the number of visits with the servals visiting the patch more than the cheetahs for all the smells except Feliway (Table 4.3, Figure 4.7). Feliway appeared to hold a similar interest for both serval and cheetah (Figures 4.6 and 4.7). There was no effect due to the interaction between smell and species for sniffs even with the control and Feliway included in the analysis (Table 4.3). However visits showed a significant interaction between species and scent type (Table 4.3). Servals visited the mouse; mint and domestic cat urine more often than the cheetahs (Figure 4.7).

Feliway was the most interesting to all the cats, except the tiger. All the females found Feliway to be of extreme interest. The smell that aroused the greatest interest for all the cats, other than Feliway, was the male cat urine. Mint, catnip and mouse (for the cheetah and the tiger) all evoked a similarly low level of interest. The male and female servals found the mouse scent to be of particular interest. The control caused the least interest of all the patches. All of the smells created significantly more interest than the control.

Figure 4.6 Mean sniffs for each species of cat in response to each scent.

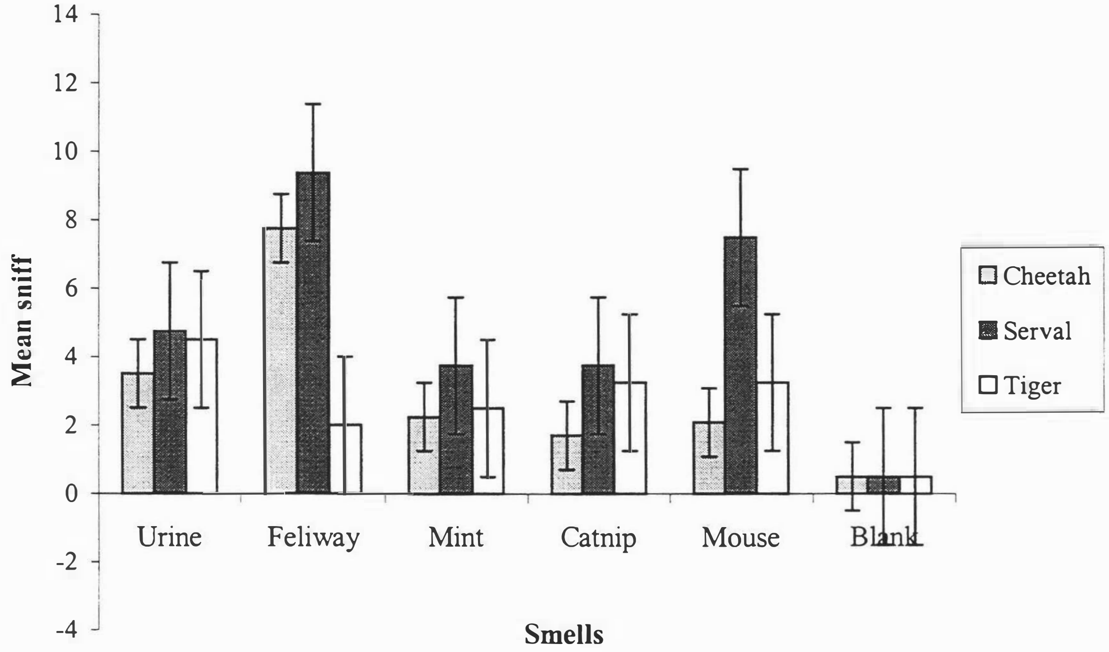
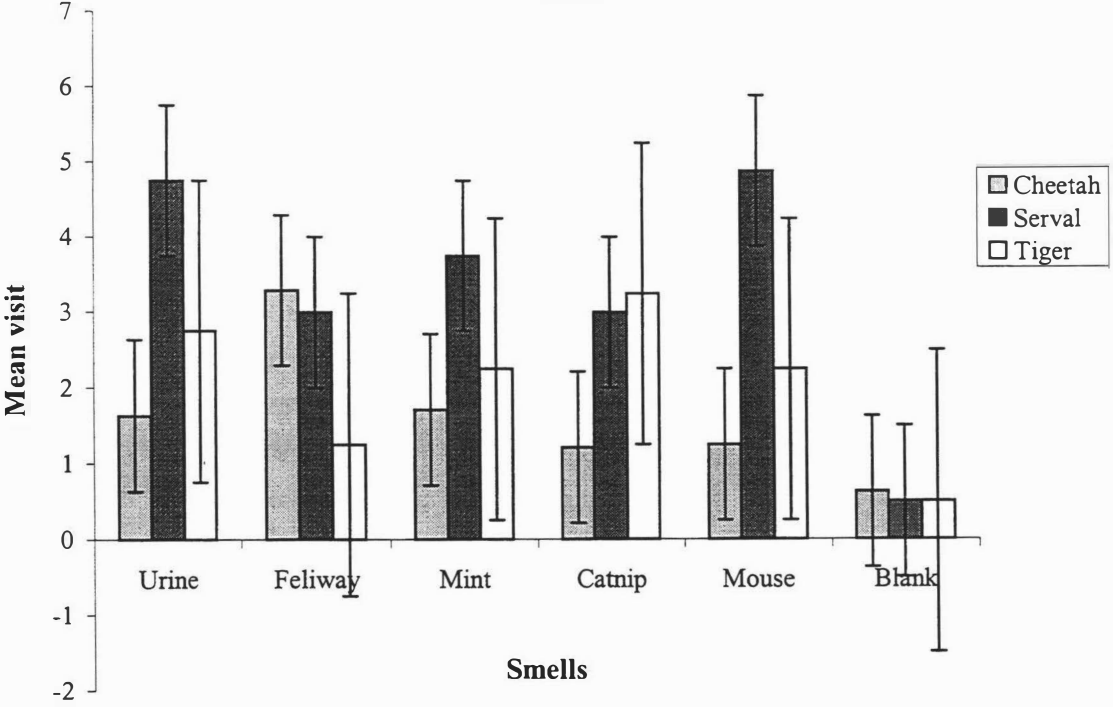


Figure 4.7 Mean number of visits by each species of cat in response to each of the scents.



4.34 Response to Catnip inside the Enclosure

The catnip extract was sprayed inside the enclosures of the lions, the serval and the tiger, Although the lions and the servals both sniffed at the areas sprayed with the catnip extract, they still did not exhibit any features of the catnip response.

The tiger had a more unusual reaction. When the catnip extract was placed inside Sam's enclosure, it was sprayed against two poles, one of these poles was frequently marked with urine, the other, much less frequently or not at all. Once the tiger was released back into his enclosure, he immediately discovered the new smell on his usual patrol around the enclosure. Once discovered, Sam began a session of intensive sniffing and licking and flehmen, which was soon followed by cheek and head rubbing against the catnip pole. This continued for nearly half and hour. Once he left the pole he moved over to an area of long grass and began rolling around on his back and then loped around in a generally playful manner. He discovered the second pole, though he spent less time sniffing this pole and then returned to rolling on his back, finally settling to rest in the sun. Overall this reaction took slightly more than an hour. As there was such a good response from Sam, the procedure was repeated about a week later, and although Sam thoroughly sniffed the marked pole, none of the other 'catnip' responses were shown again. The patches also failed to produce any reaction other than sniffing

Members of the old pride completely ignored the catnip sprayed inside their enclosure. However, they spent a great deal of time sniffing around other areas of the enclosure and the new juvenile male could be heard roaring from within the house where the lions are kept over-night.

The catnip extract was sprayed inside the enclosure of the juvenile lion 'Toby'. Although he noticed a new scent inside his enclosure, he had difficulty locating the actual scent source and spent sometime just sniffing the air, before finding the post that had been sprayed with the catnip extract. Even after locating, and investigating the catnip post Toby showed none of the behaviours associated with the catnip response.

4.4 Discussion

The different scents elicited a response from all the Felids involved in this study.

- The response to the odours was significantly greater than the control (water) for both the number of sniffs and the number of visits.
- Feliway elicited the most sniffs and the most visits.
- There was a strong sexual bias in the preference for the Feliway patches, with females approaching and sniffing the patches significantly more than males.
- The male domestic cat urine and the mouse smell elicited a good response, but were not significantly different in the number of sniffs and visits to mint and catnip.
- There was no significant difference between the species in terms of sniffing, however the servals were found to visit the scent more often than the cheetah.

4.41 Feliway

Whether the felids in this study would respond to the analogue of domestic feline facial pheromones was unknown. It is therefore interesting to see such a strong response. Out of all the species tested, the cheetah found Feliway the most interesting smell, both in terms of sniffing and visits. A strong sexual bias was found, with the females preferring the Feliway significantly more than the males. Cheetah males still favored the Feliway over the other smells offered to them, however the females' attraction to the Feliway patch was extreme in comparison. Female cheetah sniffed the Feliway patch an average of 14.75 times per presentation (15 minutes) compared to the other smells which all elicited an average of less than 5 sniffs. The females also responded by rubbing and licking against the fence where the Feliway patch was attached.

The serval female Sheba also demonstrated her attraction to Feliway sniffing it an average 15.75 times per presentation. The other smells averaged less than 7 sniffs per presentation for the servals. All female cats were observed rubbing and occasionally licking the Feliway patch. This behaviour was not observed in any of the cats for any of the other presentations.

The most common response by domestic cats to an object rubbed with feline facial secretion in the study of Verberne and De Boer (1976) was sniffing and rubbing, which is consistent with the response shown by the female cheetah and serval in this study. Flehmen occurred infrequently in the study of Verberne and De Boer (1976) and was not observed in response to the Feliway patches. The serval male seemed unimpressed with Feliway (mean sniff=3), sniffing it less than the mouse and catnip patches. The tiger was similarly uninterested, sniffing the Feliway patch the least out of all of the patches, except the control patch sprayed with water.

Verberne and De Boer (1976) found that female domestic cats showed more interest in the facial skin gland secretions collected from donor cats, than the males in their study. However, since then most of the research into feline facial pheromones has concentrated on it's ability to prevent urine marking in domestic cats (Pageat, 1997; Frank et al., 1999; Hunthausen, 2000; Mills and White, 2000). Most urine marking problems in domestic cats occur in males (Bradshaw, 1992). Therefore most of the test subjects used for studies of feline facial pheromone are male cats with urine marking problems that their owners find unacceptable. What it is that females find so interesting about these skin gland secretions and the synthetic feline facial pheromone has not yet been investigated.

Verberne and De Boer (1976) found that males and females both showed a peak interest when the female donor of the facial secretions was in oestrus. When male donor secretions were used, neither males nor females appeared to discriminate between the gender of the donor cat in terms of the length of time spent sniffing the rubbed object (Verberne and De Boer, 1976). Female domestic cats are able to discriminate better between clean objects and objects rubbed by a cat, spending more time investigating the rubbed object than males do (Verberne and De Boer, 1976).

The structure of social groups varies greatly between the different species of the Felidae, and even within a species, the social structure can be highly variable (Leyhausen, 1988). Female felids are generally more territorial, defending areas of prey abundance against intruding conspecific females. When males fight other males it is usually an issue of dominance or access to a mate (Leyhausen, 1988). Even asocial female felids will spend a large proportion of their reproductive lives living in family groups, while they raise cubs. In both the territorial context and the familial context, facial marking and facial pheromones may hold greater relevance for females than males.

It is interesting to note that the females of two of the species which showed a preference for the feline facial pheromone in this study, cheetah and serval, as well as female domestic cats, often live in close proximity to neighbouring females and generally have overlapping territories. Serval females living in the Ngorongoro Crater were found to have a 79% overlap in territorial space (Geertsema, 1985). Domestic cats and cheetah females appear to be primarily solitary, however feral domestic cats have been observed living in groups of related females, who may even share a den and raise kittens together (Macdonald et al., 1987; Leyhausen, 1988). Female cheetahs studied in the Serengeti do not associate with each other, but it is not uncommon for mothers to share, or subdivide, their territories with adult daughters (Ewer, 1973; Caro, 1994). There are numerous reports of adult female cheetah living together in other regions of Africa (Caro 1994).

Sociality may also be an explanation for the male cheetah preference for the Feliway patch. Cheetah males often form coalitions (Caro and Collins, 1987a; 1987b; Caro, 1994) and defend a territory as a group. Coalition males will rub head and face areas during greeting (Caro, 1994). Tiger and serval males are solitary and these species showed less interest in the Feliway.

Although domestic cats, servals and cheetah are not strictly social species, their more social tendency may place an increased importance on the function of facial pheromones in the social context. Unfortunately there were no females from the larger species of felid available to this study. The fact that the pheromones of the domestic cat held any interest for another species of felid is interesting. There appears to be no published literature on the pheromonal make-up of the facial secretions from any other species of felid, although there may be some family overlap in Felidae chemo-communication, particularly between species with a close phylogenetic relationship (Bininda-Emonds, et al., 2001).

Further research may look at the response of lions to the synthetic analogue of the feline facial pheromone, to determine whether the pheromones of the *Felis catus* hold any interest for *Panthera leo*, the most social of the felid species.

4.42 Urine

Overall the species, the male domestic cat urine was sniffed the most, apart from the Feliway. Although it was not statistically different from the other scents used in this study, the urine of a male domestic cat nevertheless generated a reasonable amount of interest. The cheetah, both male and female, sniffed the urine more than the other smells except Feliway. The serval showed a preference for both Feliway and the smell of mice above the urine of a male domestic cat. The tiger sniffed the domestic cat urine more than all the other odours.

In terms of visits the popularity of the cat urine is not so clear-cut. The cheetah visited the mint patches more often than the urine. The tiger visited catnip and urine patches a similar number of times. The servals visited the urine patch in proportion to the sniffs. Flehmen was not observed in any of the individuals, and no behaviours, other than sniffing, were observed in association with this smell.

There appears to be no published accounts of domestic cat urine being used as an olfactory enrichment tool for other species of felids. It is commonly known that the urine of conspecifics is of great interest for all species of carnivore (Schaller, 1972; Verberne and Leyhausen, 1976; Wemmer and Scow, 1977; Mellen, 1993; Caro, 1994). However, the response of carnivores to olfactory indications of the presence of other species of predators inside their territory is variable, some animals may ignore the presence of competitors and others may pursue or kill them (Caro, 1994). There are published anecdotal reports of carnivores being killed by other carnivores (Polis et al., 1989; Bailey, 1994; Caro, 1994; Durant, 2001).

At Orana Park none of the cats participating in the study would have encountered any smells of other carnivores inside their enclosures other than their conspecifics. Whether it is in the context of a potential predator, a potential prey, or merely as a novel animal, signs of a competitor in the vicinity may explain the interest in response to the urine.

4.43 Mouse

For the cheetah and the tiger, the mouse scent caused a similar level of interest to all the other smells except Feliway. The smell of mice did elicit a greater interest from the serval male and female than it did in the other cats. Small rodents such as rats and mice compose part of the normal diet for servals in the wild, and this may account for part of their increased interest in the smell of mice when it was presented in the patch. It is however more likely due to the fact that the servals are regularly fed the carcasses of dead rats and mice as a supplement to their normal diet. Whole carcasses are occasionally hidden inside the serval enclosure as part of the environmental enrichment program run by Orana Park. Furthermore, mice or rats have been seen to accidentally wander into the enclosures of all the felids, as they presumably do with all of the animals at the Park. For the cats, especially the servals, these occasions provide rare hunting opportunities, allowing then to exercise their hunting skills and giving them a renewed interest in their environment.

Lions are known to relish the olfactory and quite possible the tactile, joys of the dung of other animals (Powell 1995). At Orana Park the dung of the rhinoceros or the zebra was presented to the lions who would then spend an hour sniffing it and rolling in it. The sheer delight of this exercise seemed to elude both the tiger and the cheetahs who sniffed at the dung but had no further interest in it (Graeme Petrie, senior animal keeper, Orana Park, per comm.). Tigers or cheetah are both unlikely to hunt zebra or rhinoceros in their natural environment (Caro, 1994), and perhaps this accounts for their limited interest in the faeces of these species. The mouse faeces were not placed inside the serval enclosure. It may be that direct contact with the dung of their prey would cause a similar response by the serval.

4.44 Catnip (*Nepeta cataria*)

None of the felids exhibited the classic catnip reaction in response to the catnip patches, although they did approach the patch to sniff and investigate. When the catnip extract was sprayed inside the lion and serval enclosures, the animals sniffed at the areas sprayed with the catnip extract, but did not exhibit any features of the catnip response.

The tiger showed a varied response to the catnip extract when it was placed inside his enclosure. On the first occasion the tiger showed behaviours consistent with the typical catnip response (Bradshaw, 1992), however during the following presentation none of these behaviours were shown again. Perhaps the fact that the extract was sprayed over the tiger's territorial urine marks provoked a more intense interest in the catnip. Face and head rubbing is often associated with urine marking (Wemmer and Scow 1977), even rolling has been suggested as a method of scent marking (Reiger, 1979) and it may be that the behaviours seen, were a response to a new and novel smell being placed over the urine marks. However, the tiger did not urine mark in response to the catnip extract covering his own urine marks and there was a general increase in activity with the tiger bounding around his enclosure in a playful manner. If the reaction was catnip induced then why was it not repeated? Unfortunately due to the daily running of the park, it was not feasible to repeat this experiment again.

Lions have been found to be particularly sensitive to *Nepeta cataria* (Hill et al., 1976), therefore the lions at Orana Park were expected to respond to the catnip. Members of the old pride were aged between 11 and 16 years of age and completely ignored the catnip sprayed inside their enclosure, appearing not to notice it at all. However, the olfactory and auditory signs of the presence of the new juvenile male that had recently arrived at Orana Park seemed to hold a monopoly on their interest. It may be that the catnip may have been investigated if there had not been the more pressing issue of an intruder inside their territory. Hill et al. (1976) also noted that more aged individuals appeared to be less sensitive to catnip. As some members of the 'old' pride were aging individuals, this may have had an impact on their lack of response to the catnip extract sprayed inside their enclosure. When the catnip extract was sprayed inside the enclosure of the juvenile lion Toby, he showed none of the behaviours associated with the catnip response.

Some lions have already been proven to respond to catnip (Hill et al., 1976). It may be that the lion population has a genetic variance regarding the response to catnip just as the domestic cat population does (Palen and Goddard, 1966; Bradshaw, 1992). There is a relatively small genetic pool for lions in New Zealand (Graeme Petrie, senior animal keeper, Orana Park, per com.) and it may be that the gene for the catnip response is not present here.

In this study the catnip extract scented patches seemed to elicit no more interest than that of any of the other scents presented to the cats. The smell of catnip spray on the posts inside his enclosure did elicit some interesting behaviour from the tiger, however his inconsistent behaviour couldn't be explained.

4.45 Peppermint (*Mentha piperita*)

Peppermint received a similar level of interest to that shown in response to the catnip. Although there is no significant difference between the scents, peppermint appears to generate comparatively limited interest.

Peppermint extract was chosen initially as a fairly neutral smell in terms of biological imperatives. It was used in Powell (1995) and reported to have been of interest to the lions in that study. In the present study peppermint extract was also successful in eliciting a response from all of the felids participating in this study. When the peppermint patches were approached, they were sniffed but no flehmen response was observed. In Powell's study (1995) sniffing and flehmen are clumped together in the record of behaviours, so it cannot be determined whether the lions performed flehmen over the peppermint extract. However flehmen is mainly considered as sexual testing (Verberne and De Boer, 1976) and therefore may not be elicited by plant derivatives or chemical smells.

Powell did find that the incidence of back rolling increased when the various scents, especially peppermint extract, were placed inside the enclosure. No behaviour other than sniffing was seen in association with the peppermint extract patches presented to the cats at Orana Park. The fact that no back rolling behaviour was seen in this study may be attributable to the method of presentation.

Anecdotal evidence from cat breeders has indicated that peppermint essence can be used as a deterrent to domestic cats. Reis et al. (2001) found that peppermint extract was an effective repellent that stopped stock animals from feeding on crops. There is no evidence from this study to suggest that peppermint essence has any effect as a deterrent for felids. The cats did not show an excessive interest but they also did not exhibit a particular aversion to the scent.

4.46 Water

There was some reaction to the control patch. Although the overall mean number of sniffs and visits was less than one, the actual numbers varied between zero and three. Most responses to the control patch were either none or one. The fact that there was any response at all may be explained by the randomized order of the scent presentation. The animals usually received a different scent each time the patch was presented. The analysis has shown that these scents were of interest to the cats and they generally approached and sniffed the patch at least once to determine the degree to which the scent was of interest to them. The control patch was generally ignored or approached and sniffed once, discovered to be of no interest and then ignored. Only two individuals sniffed the control patch more than once. Yatima, a juvenile cheetah female, approached and sniffed the control patch more than once on two occasions. On both of these occasions the control was administered directly after the Feliway, which was so popular, especially with the females that it may have generated a hopeful enthusiasm for the patch in general, even though it had no particular scent assigned to it. The other individual to sniff the control patch more than once, was Tatu, an adult male cheetah, however there appears to be no clear reason for his return.

4.47 Different Responses

There were different responses from the felids in terms of species, gender and the individual. There was a slight difference in response between the species. The tiger was not included in the analysis because there was only one in the study. When the numbers of sniffs were considered there was no significant difference between the cheetah and the serval. However when the numbers of visits were compared, a small but significant difference was found, with the servals visiting the patch more often than the cheetah. This difference may just be a variance caused by the small number of individuals involved in the study. Alternatively it may represent a species difference in the importance of olfaction in their biology. Cheetahs primarily hunt small ungulates, such as gazelle, by sight usually during the day using the typical felid stalk and rush sequence (Caro, 1994). Servals hunt a variety of smaller prey, which they capture by waiting at the entrance to burrows for the prey to come out, or pouncing on prey in the long grass, which they appear to locate using high bounding leaps (Ewer, 1973). It may be that in the 'close quarter' style of hunting utilized by servals, scent may play a more important role.

The serval enclosure was situated in a central position within Orana Park, together with the meerkats, which are a very popular attraction with the visitors. It may be that the servals receive many more visitors than the cheetah and are therefore less shy of humans.

There was also a significant gender difference caused by the females sniffing the Feliway patch more than the males. The difference was not significant regarding the other smells, however, there seemed to be a slight inclination for the females to sniff all the patches more than the males. This trend may be influenced by the fact that the two cheetah females were still young and may still have had some youthful curiosity. Certainly the cheetah males seemed to be more reticent about showing any interest in the patches. This reticence may have been influenced by individual preferences. It is impossible to comment on the individuality of the one and only tiger and the servals, as there was only one male and one female. Any differences in response between the servals could be attributed to gender differences.

There did appear to be individual differences between the sibling cheetah males, Moja and Mbili did seem to be more wary of humans in general, including zookeeping staff. Moja and Mbili also tended to show less interest in the patch, but this may have been due to the presence of the observer. Tatu by comparison appeared more relaxed and also demonstrated more interest in the patches.

Adult cheetah male Mbili investigates the patch while keeping an eye me.

4.48 Method Review and Working with Zoos

The problems that were found with the methods of this study and the challenges that arose from working in a zoo, which has a commercial focus, overlapped and so they will be discussed together.

Observing the cats outside of the presentation times may have given an indication of the effects of the novel scents on the behaviour of the felids. However it would have been impossible to separate the effects of the patch, from the effects of the enrichment techniques used by Orana Park, or any other aspects of park environment that may have impacted on the animals' behaviour. Occasionally the park environmental enrichment program would coincide with the presentation of the scented patches in this study. At such times the observations could not be continued.

The enrichment procedures carried out by the zookeepers, that involved placing novel substances inside the enclosures of the felids appeared to create more interest from the felids than the patches attached to the outside of the fence. The felids' response to the catnip extract sprayed inside the enclosure appeared to be consistent with this trend. However it wasn't feasible to place the scents used in this study inside the enclosures, as it would have required considerable zookeeper cooperation. The park had had previously bad experiences cooperating with students attempting studies. Hence the Orana Park management committee was reluctant to commit keeper time to any study.

Placing novel substances inside the enclosure may place a greater importance on the tactile and visual aspects of the presentation, over the olfactory aspects. These features may contribute to the overall impact of the enrichment technique. The tiger was given an old tyre that had been kept in the lions' nighttime enclosure. The tiger was observed to spend over an hour playing, chewing, rolling and clawing with this tyre. The combination of olfactory and tactile stimulation and the ability to directly interact with the stimulus, in an area that the animal is accustomed to may have greatly enhanced its attractiveness. However much of the tyre's attractiveness must be attributed to its stimulating olfactory properties, as the tyres that are kept permanently inside the tigers enclosure attracted no such attention. A better indication of any olfactory preferences might have been established if the scents were applied to such an object and presented inside the enclosures.

A further complication that comes from working with zoos is the small numbers of animals that can be studied. These animals may not be used to observers and in this case there did appear to be a response to the presence of the observer, with some individuals spending more time observing the observer, than they did investigating the patch. Yet on other occasions some individuals appeared to have no interest in either the observer or the patches. The zookeepers usually visited all the cats in the morning to check on them. The arrival of the keeper's truck almost invariably caused the cats to get up and begin pacing along the fence, however on some days even the arrival of a keeper was not enough to motivate the cat to get up and investigate. This appears to be a problem that is not restricted to the animals at Orana Park. Hill et al. (1976) found that the cheetahs intended to be included in their study simply would not approach the fence to investigate their catnip boxes. In these circumstances it is difficult to say exactly what the cats are responding to or not responding to, whether the patches are just not interesting to that individual or whether that individual is just not interested.

As previously discussed regarding the response to the blank control patches, there may have been some overlap between the effect of the patches that generated more interest and those patches that followed. The presentation of the patches was randomized to prevent habituation to the scents. However the response to the Feliway by the females was so enthusiastic that there may have been some flow on effects. Further research into the responses of other felid species towards the synthetic feline facial pheromones may be warranted. If this pheromone proves to have a calming effect on other species of large felids this may be extremely useful in the zoo environment, although the cost of this product may be prohibitive.

4.49 Conclusions

The effectiveness of these scents in terms of environmental enrichment appears to be marginal in this study. The response to all of the scents, as determined by the number of sniffs and the number of visits was significantly greater than the response to the control. The urine and the mouse scent elicited a good response, but were not significantly different to the amount of sniffs and visits mint and catnip received.

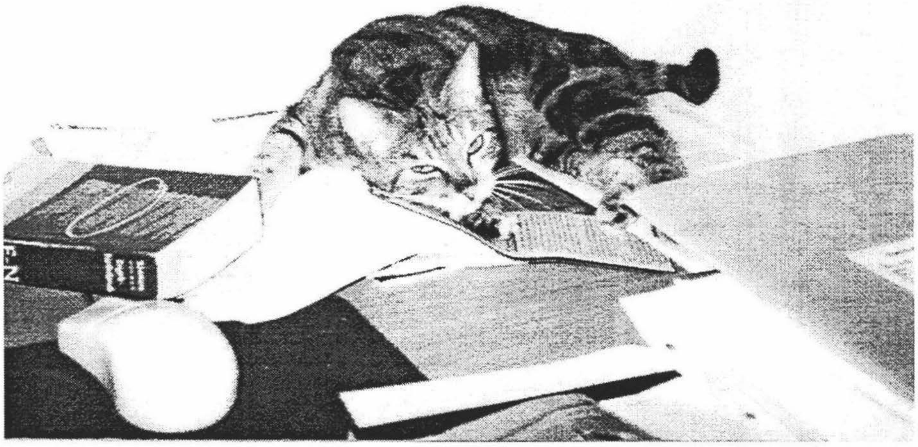
All of the females in this study (cheetah (*Acyninox jubatus*) and serval (*Felis serval*)) showed the greatest interest in the Feliway patches, and responded by sniffing and rubbing enthusiastically. The response to the Feliway was significantly greater than the responses to the other scents in this study, although the males' response to the Feliway was not as animated as the females'. Feliway is a commercially produced synthetic analogue of the facial pheromones of the domestic cat.

Zoos can be difficult environments in which to conduct research. Small population numbers and the uncontrollable conditions that arise from being a public facility with abundant environmental variance can mean that conclusive results may be difficult to attain. The need for keeper cooperation in order to carry out observations can cause inconvenience to the zoo and to the researcher.

In this case the value of these scents as a tool for environmental enrichment may need further investigation. Feliway may hold the greatest potential, as it received considerable attention from the females in this study. Like many other environmental enrichment techniques, olfactory stimulation may be most effective when it is used in conjunction with other methods of environmental enrichment.

Chapter Five

Thesis Summary



Asia assists with the paperwork (photo by Karin Weidgraaf)

"A man has to work so hard so that something of his personality stays alive. A tomcat has it so easy, he only has to spray and his presence is there for years on rainy days."

Albert Einstein

5.1 Thesis Summary

The realm of olfactory sensations, perception and communication is a world of information filled with subtle suggestions, urgent demands and tempting entreaties that humans are largely unaware of. Yet even as olfactory paupers, humans are endlessly fascinated with scent as demonstrated by the many cultural rituals involving scent all over the world (Watson, 2000). The author H.G. Wells was reputedly exceptionally attractive to women. When his friend Somerset Maugham wondered at the cause of Well's success, admirers all told him the same thing. It was the way H.G. Wells smelled.

The importance of odour in the mammalian world has also not escaped our notice. The Sanskrit name for tiger is *vyagra,* derived from a verb root meaning 'to smell' and it is likely that the extreme persistence of the tigers' scent marks may have been the inspiration for this. Even in monsoon weather the tigers scent marks can last for weeks (Brahmachary and Dutta, 1981).

To be effective, environmental enrichment must be applicable to the sensory world of the animal and to the quantity of information required to survive in the wild (Poole, 1998). As of yet, relatively little work as focused on the possibilities of olfactory stimulation as an environmental enrichment technique for captive carnivores. Powell (1995) observed that various odours including allspice, almond and peppermint essence increased the occurrence of sniffing and flehmen behaviours.

The environmental enrichment potentials of various scents were investigated at Orana Wildlife Park in Christchurch. Six cheetahs, two servals and one tiger were exposed to scented patches. The scents used were catnip, peppermint, mouse faeces, urine from an entire male domestic cat and a synthetic analogue of the facial pheromones (Feliway) of the domestic cat. Although all of the scents elicited responses from the cats that were significantly greater than the responses to the control, these responses were still brief on the whole (Appendix 4). The synthetic feline facial pheromone was the exception. All of the females in this study (two cheetahs and one serval) responded enthusiastically to the

Feliway, sniffing this scent significantly more than the other odours, and rubbing against the scent patch. None of the other animals showed this level of response to the feline facial pheromones. The responses from the felid females at Orana Park infer that there may be some environmental enrichment potential in the use of synthetic feline facial pheromones. The other scents elicited a limited interest, although their usefulness as tools for environmental enrichment should perhaps not be extrapolated from the results in this study. There were problems with the methods of this study and difficulties that arose from working with a zoo. The small number of animals, conflicts with an already established program of environmental enrichment, and the lack of environmental control present in a public institution render the results dubious. Some of the animals were comfortable with people and would approach the scents, others such as the male cheetahs were extremely wary and may have shown less response because of the presence of an observer. Different methods of presentation may have elicited a greater response from the cats but would have required considerable keeper interaction.

Although the response to the domestic cat urine was small, the cheetah were observed to spend an hour or more investigating each others urine marks on the days when animals were moved into different enclosures. Therefore the environmental enrichment potential of conspecific urine was investigated using domestic cats at the Heinz Watties Feline Nutrition Research Unit, a research unit of Massey University, Palmerston North, New Zealand as models for wild felids living in captivity. The use of conspecific urine as environmental enrichment could provide complexity and novelty and elicit a wider range of behaviours. Ten anoestrous females were presented with combinations of urine samples collected from four entire male domestic cats with an established social dominance order. The latency, number of visits, number of flehmen responses and the sniff duration were recorded as measures of female interest in the urine of each male. The anoestrous females showed a limited response. They responded to the urine of all four males in a similar manner. Some females shared varying degrees of relatedness with two of the males, however this did not appear to influence their response.

Females are known to spend more time investigating the urine when they are in oestrus (Bradshaw, 1992). Urine samples from the four entire males were presented to eighteen oestrous females. In oestrus, females were observed to discriminate between the urine samples of the different males in terms of their sniff duration, number of visits and number of flehmen responses. Overall the urine of Sunny, the most dominant, heaviest and oldest male was preferred over the other males. Bransen's urine was the least preferred and received significantly less visits and shorter duration of sniffing. The urine of the two males that are not related to any of the females, Brock and Titan, received intermediate levels of attention from the females. These two males also held intermediate dominance ranks, below Sunny but above Bransen.

When the responses of the females towards the two males that had varying degrees of relatedness with the females in the group were analysed, a strong relationship effect was revealed. The higher the degree of relatedness the lower the sniff duration, number of flehmen and number of visits to that male's urine samples. The effect of relatedness was stronger for Sunny than it was for Bransen. Females that are unrelated, or only slightly related to Sunny showed more interest in his urine than females that are unrelated, or slightly related to Bransen. When the effect of relatedness is taken into account Sunny's urine is even more popular.

Sunny is not only the most dominant, oldest and heaviest male, he has also had the most breeding experience. However, although this may account for preferences amongst the eight 'breeding' females, it cannot account for the attention that his urine received from the naïve females. Amongst the females that were highly related to Sunny some interesting consistencies emerged. Some females appeared to consistently prefer the urine from Brock while others preferred the urine from Titan. Unfortunately there was not enough data to adequately analyze these preferences.

Mating experience may account for some of the female preferences. The females Kola and Tori preferred Sunny's urine despite the fact that they were closely related to him. All of the other females that shared a similar level of relatedness to Sunny showed preferences for other males. Kola and Tori had both been bred with Sunny. In comparison, Kola's full sister, Fya had only been mated with Titan and showed a consistent preference for Titan's urine in all of the parameters.

Female domestic cats were conventionally thought to accept any male that courts her (Bradshaw, 1992) and for rural or feral living females, mating with any male may be better than failing to reproduce due to the lack of a preferred mate. The results of this study of the responses of oestrous females to the urine of entire males infer that females can discriminate between the urine of males on the basis of relatedness as well as male dominance. These results are consistent with the findings of Ishida et al. (2001) that female domestic cat will avoid mating with males to whom they are closely related.

There does not appear to be much difference between the responses of oestrous females and anoestrous females in terms of their average sniff duration or average number of visits (Appendix 1 and Appendix 3). The latency to approach the males' urine samples appears to be slightly shorter for oestrous females (Figure 2.5 and 3.3). The anoestrous and oestrous data cannot be directly compared, as they are different experiments and changes in female response can only be implied.

Whether the selective responses of oestrous female to male urine are representative of female mate choice is unknown, however indications that females show any kind of discrimination may warrant further attention to this area of felid lives. Evidence of female choice may have been overlooked in the presence of male dominance struggles and male monopolization of oestrous females. There is ample anecdotal evidence that females will consistently mate with certain males, apparently irrespective of the social dominance ranking (Leyhausen, 1979; Natoli et al., 2001).

Courtship and mating patterns are very similar amongst the Felidae, conforming in general to those of the domestic cat (Ewer, 1973). Oestrous lionesses have been observed to avoid pride males and mate instead with nomadic or neighbouring males (McNeice et al. 2001). Lindburg and Fitch-Snyder (1994) suggest that the phenomenon of mate choice in the Felidae may be more wide spread than previously realized and it must be considered as affecting outcomes in many captive-breeding situations.

The circumstances under which any aspect of felid reproduction takes place are drastically altered in the captive environment. Captive animals have no opportunities for mate location or mate choice, as zoo managers stage all pairings. The failure of any species to reproduce at levels that are comparable to those of its wild counterpart directly implicates inadequacies in the captive environment (Lindburg and Fitch- Snyder, 1994). There are ample enrichment opportunities to provide the requisite environmental conditions and species specific information to address this instinctive drive.

It has long been accepted that even the most solitary species have social interactions with familiar animals that are part of their ‘solitary’ community, by leaving smelly signs to inform one another of their activities (Leyhausen, 1965). Lindburg and Fitch- Snyder (1994) suggest that where solitary species are appropriately accommodated, failure to reproduce may be a result of the way in which mating pairs are brought together. Olfactory stimulation from a potential mate before the pair is introduced had resulted in improved mating success in captive cheetah (Lindburg et al., 1993).

Olfactory stimulation from potential mates may also have enrichment benefits for animals that are not in captive breeding programs, as the instinctual drive to search for mates can lead to frustration and stress (Carlstead and Seidensticker, 1991).

As a tool for the environmental enrichment of oestrous females, conspecific urine still appears to be limited in its capacity to hold an animal’s attention. Responses from oestrous domestic cats were minimal overall with females averaging less than five seconds of sniffing at the urine samples and visiting less than once per three minute observation period, results that are quite similar to the overall response of the females in anoestrous. Responses varied from zero to sixty-two seconds sniff duration (Appendix 3). The comparatively small response may be in part due to the fact that the urine is excreted urine, not sprayed urine. The females may also have habituated to the odour of the males after several exposures. However, the discriminating responses of the oestrous females implies that male urine holds information of interest to other animals. Conspecific urine may be a potentially useful tool for environmental enrichment but further research is needed.

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“No one can have experienced to the fullest, the true sense of achievement and satisfaction who has never pursued and successfully caught his tail”

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Appendices

“Oh cat; I’d say, or pray: be-ootiful cat! Delicious cat! Exquisite cat! Satiny cat! Cat like a soft owl, cat with paws like moths, jewelled cat, miraculous cat! Cat, cat, cat,cat.”

Dorris Lessing

Appendix 1.

Anoestrus sniff duration (seconds) followed by the mean, standard deviation and the standard error.

Dusky

Trial	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*mean*	*s.d.*	*s.e.*
Sunny	4	4	9	0	1	6	5	5	9	0	6	8	6	2	2	2.4	3.0	0.8
Brock	0	5	2	2	1	14	2	10	0	0	0	3	0	4	0	3.1	4.1	1.1
Titan	0	0	1	3	15	3	2	4	0	0	1	15	3	4	6	3.3	4.9	1.3
Bransen	6	5	2	0	3	3	3	0	0	3	0	11	3	4	4	1.9	2.9	0.7

Kola

Trial	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*mean*	*s.d.*	*s.e.*
Sunny	0	5	4	6	0	5	2	0	0	5	11	2	5	3	7	2.5	3.1	0.8
Brock	0	0	0	0	7	0	0	3	0	5	1	2	0	3	0	1.5	2.2	0.6
Titan	0	0	0	3	6	4	0	3	0	3	6	0	1	0	0	1.9	2.3	0.6
Bransen	3	6	0	0	0	1	0	0	0	0	0	0	0	0	2	1.2	1.7	0.4

Mirrim

Trial	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*mean*	*s.d.*	*s.e.*
Sunny	38	5	5	0	0	0	4	6	0	12	9	2	9	0	5	5.7	9.6	2.5
Brock	0	0	0	1	11	0	0	4	0	9	0	11	0	0	0	2.6	4.3	1.1
Titan	0	0	6	5	3	5	0	0	4	0	4	4	0	7	2	2.2	2.5	0.7
Bransen	10	5	4	0	2	0	2	4	5	6	1	1	3	1	2	2.1	2.7	0.7

Sheba

Trial	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*mean*	*s.d.*	*s.e.*
Sunny	7	3	11	6	3	0	2	2	14	5	13	9	9	28	0	5.2	7.2	1.9
Brock	0	3	3	1	0	1	10	23	2	2	5	9	0	0	4	4.2	6.1	1.6
Titan	7	6	2	4	3	1	0	0	1	0	4	13	6	0	6	2.8	3.6	0.9
Bransen	18	16	2	2	23	10	3	6	0	0	5	13	5	1	0	6.0	7.4	1.9

Wren

Trial	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*mean*	*s.d.*	*s.e.*
Sunny	5	0	4	5	5	6	0	2	0	4	16	3	0	2	0	2.8	4.1	1.1
Brock	4	6	3	0	13	1	8	4	1	1	0	2	0	4	7	3.4	3.7	0.9
Titan	1	8	3	0	8	5	0	0	2	0	9	1	3	0	3	2.5	3.2	0.8
Bransen	0	0	1	0	9	0	0	1	1	5	0	0	0	0	1	1.5	2.5	0.6

Fya

Trial	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*mean*	*s.d.*	*s.e.*
Sunny	0	4	7	7	0	2	0	0	6	8	4	2	5	2	0	2.5	2.9	0.8
Brock	0	0	5	2	6	0	3	1	2	0	3	0	0	0	2	1.6	2.0	0.5
Titan	5	2	0	0	0	5	1	2	0	0	1	3	3	0	8	1.9	2.4	0.6
Bransen	0	3	3	3	0	14	2	0	0	9	0	0	5	3	1	2.7	4.0	1.0

Milo

Trial	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*mean*	*s.d.*	*s.e.*
Sunny	0	0	0	4	0	0	0	0	0	5	0	0	0	0	0	1.0	1.6	0.4
Brock	0	5	3	0	0	0	0	0	0	0	0	0	0	0	0	0.6	1.5	0.4
Titan	3	4	0	0	0	7	0	0	2	0	3	0	3	0	0	1.8	2.1	0.6
Bransen	6	1	6	3	3	0	0	0	0	0	0	0	0	0	2	1.7	2.2	0.6

Bella

Trial	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*mean*	*s.d.*	*s.e.*
Sunny	11	0	4	7	2	1	2	2	9	4	0	0	3	0	6	2.7	3.5	0.9
Brock	0	10	4	7	1	2	9	4	2	0	9	0	0	2	3	3.6	3.6	0.9
Titan	3	18	6	0	2	5	1	2	0	4	4	3	4	0	4	2.5	4.4	1.1
Bransen	7	12	1	3	4	3	0	0	0	7	0	10	2	0	0	3.2	4.0	1.0

Nikki

Trial	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*mean*	*s.d.*	*s.e.*
Sunny	19	18	30	0	43	12	16	16	31	11	32	16	20	6	3	8.8	11.7	3.0
Brock	0	20	5	13	13	12	2	9	0	0	0	2	0	2	5	5.6	6.4	1.6
Titan	0	10	8	6	0	18	8	0	3	3	0	14	0	0	4	4.6	5.7	1.5
Bransen	13	6	1	8	11	5	4	5	7	0	0	26	0	0	0	4.9	7.0	1.8

Tori

Trial	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*mean*	*s.d.*	*s.e.*
Sunny	37	8	13	6	0	17	6	26	18	3	9	11	3	13	0	7.5	10.1	2.6
Brock	5	1	3	11	0	12	0	12	1	4	2	6	0	0	3	4.1	4.4	1.1
Titan	3	6	0	3	0	4	5	0	0	0	0	0	7	0	0	2.2	2.6	0.7
Bransen	2	7	2	3	0	4	0	0	0	0	1	2	0	0	2	1.5	2.0	0.5

Appenidx 1 continued. Number of anoestrus visits followed by the mean, standard deviationand the standard error.

Dusky

Trial	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*mean*	*s.d.*	*s.e.*
Sunny	1	1	1	0	1	2	2	1	1	0	1	2	2	1	1	1.13	0.64	0.17
Brock	0	1	1	1	1	1	1	1	0	0	0	2	0	0	1	0.67	0.62	0.16
Titan	0	0	1	1	1	1	1	2	0	0	1	1	1	1	1	0.8	0.56	0.14
Bransen	1	1	2	2	1	1	2	0	0	1	0	2	1	1	1	1.07	0.7	0.18

Kola

Trial	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*mean*	*s.d.*	*s.e.*
Sunny	1	1	1	2	0	1	1	0	0	1	1	1	2	1	1	0.93	0.59	0.15
Brock	0	0	0	0	1	0	0	1	0	1	1	1	0	0	1	0.4	0.51	0.13
Titan	0	0	0	0	1	1	0	1	0	1	1	0	1	0	0	0.4	0.51	0.13
Bransen	1	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0.27	0.46	0.12

Mirrim

Trial	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*mean*	*s.d.*	*s.e.*
Sunny	1	1	1	0	0	0	1	1	0	1	1	1	1	0	2	0.73	0.59	0.15
Brock	0	0	0	1	2	0	0	1	0	1	0	1	0	0	0	0.4	0.63	0.16
Titan	0	0	1	1	1	1	0	0	1	0	1	1	0	1	2	0.67	0.62	0.16
Bransen	1	1	1	0	1	0	1	1	2	1	1	1	1	1	1	0.93	0.46	0.12

Sheba

Trial	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*mean*	*s.d.*	*s.e.*
Sunny	1	1	3	1	1	0	1	1	1	1	1	1	1	3	0	1.13	0.83	0.22
Brock	0	1	1	1	0	1	2	1	1	1	2	1	0	2	0	0.93	0.7	0.18
Titan	1	1	2	2	1	1	0	0	1	0	1	1	1	0	1	0.87	0.64	0.17
Bransen	1	2	1	2	1	1	1	1	0	0	1	2	1	1	0	1	0.65	0.17

Wren

Trial	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*mean*	*s.d.*	*s.e.*
Sunny	2	1	2	1	1	3	0	1	0	1	2	1	0	1	0	1.07	0.88	0.23
Brock	1	1	1	0	3	1	3	1	1	1	0	1	0	1	1	1.07	0.88	0.23
Titan	1	2	1	0	3	2	0	1	1	0	2	1	1	0	1	1.07	0.88	0.23
Bransen	1	0	1	0	3	0	0	1	1	1	0	0	0	0	1	0.6	0.83	0.21

Fya

Trial	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*mean*	*s.d.*	*s.e.*
Sunny	1	1	2	1	0	1	0	0	1	2	1	1	1	1	0	0.87	0.64	0.17
Brock	0	0	1	1	2	0	1	1	1	0	1	0	0	1	0	0.6	0.63	0.16
Titan	1	1	0	0	0	1	1	1	0	0	1	1	1	0	1	0.6	0.51	0.13
Bransen	0	1	1	1	0	2	1	0	0	1	0	0	2	1	1	0.73	0.7	0.18

Milo

Trial	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*mean*	*s.d.*	*s.e.*
Sunny	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0.13	0.35	0.09
Brock	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0.13	0.35	0.09
Titan	1	1	0	0	0	1	0	0	1	0	1	0	1	0	0	0.4	0.51	0.13
Bransen	1	1	1	1	1	0	0	0	0	0	0	0	0	0	1	0.4	0.51	0.13

Bella

Trial	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*mean*	*s.d.*	*s.e.*
Sunny	1	0	2	2	1	1	1	1	1	1	0	0	1	0	2	0.93	0.7	0.18
Brock	0	2	1	1	1	1	2	1	1	0	1	0	0	1	1	0.87	0.64	0.17
Titan	1	2	2	0	1	1	1	1	0	2	1	1	1	0	2	1.07	0.7	0.18
Bransen	1	2	1	1	1	1	0	0	0	2	0	1	1	0	0	0.73	0.7	0.18

Nikki

Trial	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*mean*	*s.d.*	*s.e.*
Sunny	1	2	3	0	1	2	1	2	2	2	2	1	2	2	1	1.6	0.74	0.19
Brock	0	1	1	2	2	1	1	2	0	0	0	1	0	1	1	0.87	0.74	0.19
Titan	0	1	1	1	0	1	1	0	1	1	0	3	0	0	1	0.73	0.8	0.21
Bransen	1	1	1	1	2	1	1	1	1	0	0	3	0	0	0	0.87	0.83	0.22

Tori

Trial	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*mean*	*s.d.*	*s.e.*
Sunny	2	1	2	1	0	2	2	1	2	1	1	2	1	1	0	1.27	0.7	0.18
Brock	1	2	1	1	0	1	0	2	1	2	1	2	0	1	0	1	0.76	0.2
Titan	1	1	0	0	0	1	1	0	0	0	0	0	2	0	0	0.4	0.63	0.16
Bransen	2	2	1	1	0	1	0	0	0	0	1	1	0	0	1	0.67	0.72	0.19

Appenidx 1 continued. Anoestus latency to approach the urine samples followed by the mean, standard deviation and the standard error. Dashes show a failure to approach the urine sample.

Dusky

Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	mean	s.d.	s.e.
Sunny	3	18	4	-	88	9	1	7	26	-	36	12	52	17	14	22.1	24.5	6.32
Brock	-	115	22	12	3	102	4	20	-	-	-	5	-	12	-	32.8	43.6	11.2
Titan	-	-	68	31	2	5	52	50	-	-	29	32	9	3	6	26.1	23.1	5.96
Bransen	168	7	6	-	3	15	18	-	-	6	-	14	2	95	5	30.8	52.6	13.6

Kola

Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	mean	s.d.	s.e.
Sunny	-	2	42	14	-	34	2	-	-	23	18	16	8	16	56	21	16.8	4.35
Brock	-	-	-	-	4	-	-	118	-	6	33	5	-	-	-	33.2	48.9	12.6
Titan	-	-	-	-	34	3	-	9	-	61	8	-	27	-	-	23.7	21.9	5.66
Bransen	9	9	-	-	-	12	-	-	-	-	-	-	-	-	12	10.5	1.73	0.45

Mirrim

Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	mean	s.d.	s.e.
Sunny	30	4	13	-	-	-	4	11	-	18	14	78	4	-	13	18.9	22.2	5.73
Brock	-	-	-	20	13	-	-	8	-	24	-	19	-	-	-	16.8	6.3	1.63
Titan	-	-	16	7	25	10	-	-	6	-	7	7	-	37	16	14.6	10.5	2.71
Bransen	13	2	22	-	6	-	66	16	15	9	11	15	53	36	14	21.4	19	4.9

Sheba

Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	mean	s.d.	s.e.
Sunny	34	20	6	116	31	-	3	9	29	39	95	18	56	17	-	36.4	34.2	8.83
Brock	-	115	70	41	-	10	10	20	74	118	44	2	-	-	116	56.4	45	11.6
Titan	11	12	16	8	2	30	-	-	11	-	116	3	16	-	1	20.5	32.7	8.44
Bransen	6	6	7	11	5	5	67	112	-	-	57	12	12	55	-	29.6	34.9	9.01

Wren

Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	mean	s.d.	s.e.
Sunny	16	6	2	2	16	13	-	8	-	16	14	10	-	28	-	11.9	7.52	1.94
Brock	12	9	93	-	1	83	5	12	6	8	-	22	-	6	23	23.3	31	8
Titan	9	3	11	-	4	8	-	10	10	-	7	8	3	-	35	9.82	8.82	2.28
Bransen	-	-	8	-	1	-	-	55	42	10	-	-	-	-	3	19.8	22.8	5.89

Fya

Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	mean	s.d.	s.e.
Sunny	-	11	16	9	-	8	-	-	16	4	12	8	6	9	-	9.9	3.93	1.01
Brock	-	-	10	3	18	-	80	24	50	-	9	-	-	-	23	27.1	25.7	6.64
Titan	13	48	-	-	-	10	6	6	-	-	10	4	90	-	13	22.2	28.7	7.41
Bransen	-	56	10	12	-	5	3	-	-	97	-	-	11	9	26	25.4	31.4	8.1

Milo

Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	mean	s.d.	s.e.
Sunny	-	-	-	8	-	-	-	-	-	13	-	-	-	-	-	10.5	3.54	0.91
Brock	-	14	6	-	-	-	-	-	-	-	-	-	-	-	-	10	5.66	1.46
Titan	38	10	-	-	-	19	-	-	9	-	73	-	15	-	181	49.3	62.3	16.1
Bransen	24	58	16	88	5	-	-	-	-	-	-	-	-	-	11	33.7	32.5	8.39

Bella

Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	mean	s.d.	s.e.
Sunny	48	-	2	1	5	2	23	2	55	66	-	-	86		6	26.9	31.2	8.05
Brock	-	10	52	1	6	1	1	2	7	-	15	-	-	2	15	10.2	14.9	3.84
Titan	1	2	2	-	16	45	3	38	-	2	88	5	28	-	1	19.3	26.7	6.9
Bransen	2	9	50	12	5	3	-	-	-	14	-	73	21	-	-	21	24.4	6.3

Nikki

Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	mean	s.d.	s.e.
Sunny	66	41	5	-	4	10	2	1	28	5	6	60	7	9	10	18.1	21.9	5.66
Brock	-	16	66	25	18	6	24	4	-	-	-	24	-	22	45	25	18.3	4.73
Titan	-	26	29	12	-	6	108	-	16	25	-	3	-	-	17	26.9	31.7	8.18
Bransen	15	48	41	24	18	12	27	26	36	-	-	20	-	-	-	26.7	11.7	3.01

Tori

Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	mean	s.d.	s.e.
Sunny	20	2	4	16	-	7	1	7	18	14	27	12	59	16	-	15.6	15.1	3.9
Brock	17	-	13	6	-	9	-	5	57	5	5	-	-	-	9	14	16.6	4.3
Titan	13	14	-	10	-	18	15	-	-	-	-	-	8	-	181	37	63.6	16.4
Bransen	5	6	4	19	-	7	-	-	-	-	58	10	-	-	116	28.1	39.8	10.3

Appendix 2. The dates when each female cat was observed in oestrus

Month	Dusky	Kola	Bella	Fya	Sheba	Nikki	Wren	Tori
November	24/11/00	24/11/00	24/11/00					
			25/11/00					
January	11/1/01							
	17/01/01	17/01/01			17/01/01	17/01/01		
		19/01/01	19/01/01	19/01/01	19/01/01	19/01/01		
		20/01/01	20/01/01	20/01/01	20/01/01	20/01/01		
		21/01/01	21/01/01	21/01/01	21/01/01	21/01/01	21/01/01	
February		23/02/01		23/02/01				23/02/01
	27/02/01		27/02/01	27/02/01		27/02/01		27/02/01
	28/02/01	28/02/01	28/02/01	28/02/01				28/02/01
March								
			14/03/01					
			15/03/01					
		16/03/01	16/03/01					
			17/03/01					
		19/03/01		19/03/01				
		21/03/01		21/03/01				
		22/03/01		22/03/01				
				23/01/01				
		26/03/01		26/03/01	26/03/01	26/03/01		
				27/03/01	27/03/01	27/03/01		
	28/03/01			28/03/01	28/03/01			
	30/03/01		30/03/01		30/03/01			
April					1/4/01			
	2/4/01				2/4/01			
					3/4/01			3/4/01
								5/4/01
								6/4/01
	15/04/01				15/04/01	15/04/01		15/04/01
					16/04/01	16/04/01		16/04/01
					17/04/01	17/04/01		
						18/04/01		
	23/04/01							
May	1/5/01							
							11/5/01	
							13/05/01	13/05/01
	14/05/01							14/05/01
	15/05/01						15/05/01	
	17/05/01						16/05/01	
								17/05/01

Appendix 2 (continued). The dates when each female cat was observed in oestrus

Month	Nui	Ziggy	Chyna	Astra	Asia	Pippe	Ngaio	Suede	Muffy	Broom
November										
January										
February		23/02/01								
						27/02/01				
March										
		14/03/01		14/03/01						
		15/03/01		15/03/01						
				16/03/01						
				17/03/01						
				18/03/01						
				19/03/01						
				20/03/01						
							21/03/01			
						22/03/01	22/03/01		22/03/01	
						23/01/01	23/01/01		23/01/01	
						26/03/01				
						27/03/01				
						28/03/01				
						30/03/01				
April										
			2/4/01							
			3/4/01					3/4/01	3/4/01	3/4/01
			5/4/01		5/4/01		5/4/01	5/4/01	5/4/01	5/4/01
			6/4/01				6/4/01		6/4/01	6/4/01
			7/4/01				7/4/01		7/4/01	
	12/4/01									
	13/04/01			13/04/01		13/04/01				
	15/04/01	15/04/01		15/04/01		15/04/01			15/04/01	15/04/01
		16/04/01		16/04/01		16/04/01	16/04/01		16/04/01	16/04/01
		17/04/01			17/04/01		17/04/01		17/04/01	17/04/01
					18/04/01					
					19/04/01					
			20/04/01		20/04/01					
			21/04/01							
			22/04/01							
		30/04/01		30/04/01		30/04/01				30/04/01
May		1/5/01		1/5/01		1/5/01			1/5/01	2/5/01
		2/5/01								
		3/5/01								
							10/5/01			
	11/5/01		12/5/01				11/5/01			
	13/05/01				13/05/01		13/05/01	13/05/01	13/05/01	13/05/01
	14/05/01		14/05/01		14/05/01		14/05/01	14/05/01	14/05/01	14/05/01
	15/05/01		15/05/01		15/05/01			15/05/01	15/05/01	15/05/01
		16/05/01	16/05/01							
		17/05/01						17/05/01		
		18/05/01								

APPENDIX 3

Oestrus sniff duration (seconds) followed by the mean, standard deviation and the standard error.

	Dusky																	Dusky	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	9	3	6	16	6	7	11	1	5	4	2	10	0	6	3	3	5.75	4.16	1.04
Titan	4	6	1	1	0	2	2	0	3	3	7	1	5	5	5	7	3.25	2.38	0.60
Brock	8	0	2	2	8	2	6	0	2	13	2	0	3	0	2	25	4.69	6.53	1.63
Bransen	0	1	1	1	1	1	0	3	1	0	0	12	2	4	1	1	1.81	2.93	0.73

	Fya																		Fya	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	mean	s.d.	s.e.
Sunny	0	2	3	3	0	0	16	2	0	0	0	2	0	3	0	2	0	1.94	3.83	0.93
Titan	5	6	1	0	1	17	3	6	4	2	0	0	0	0	14	4	11	4.35	5.17	1.25
Brock	0	2	0	5	2	3	1	0	0	6	5	0	0	1	2	7	0	2.00	2.37	0.58
Bransen	4	0	0	0	4	4	0	0	1	0	0	0	2	0	0	0	0	0.88	1.58	0.38

	Sheba																	Sheba	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	10	3	10	11	6	39	6	17	0	2	4	6	0	1	9	5	8.06	9.43	2.36
Titan	2	6	10	0	4	7	8	2	1	0	0	0	4	1	8	2	3.44	3.37	0.84
Brock	1	0	6	0	9	9	0	6	16	9	17	0	1	9	2	0	5.31	5.75	1.44
Bransen	2	2	2	0	2	3	4	2	2	4	2	1	0	3	1	1	1.94	1.18	0.30

	Bella																	Bella	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	0	0	3	9	0	0	4	0	0	0	6	5	7	0	0	0	2.13	3.10	0.77
Titan	0	9	6	0	3	7	0	11	4	2	5	0	5	0	0	0	3.25	3.62	0.91
Brock	3	0	0	0	3	2	8	4	1	0	3	17	6	3	0	3	3.31	4.32	1.08
Bransen	2	0	1	0	0	2	6	2	1	0	6	6	0	1	0	1	1.75	2.24	0.56

	Nikki																	Nikki	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	19	0	17	24	18	10	14	7	0	19	12	0	13	0	7	9	10.6	7.81	1.95
Titan	1	14	2	0	1	12	4	1	4	15	0	0	0	0	2	0	3.50	5.24	1.31
Brock	0	22	12	5	1	14	25	3	2	0	7	5	1	0	0	0	6.06	8.06	2.02
Bransen	0	10	0	0	3	0	8	17	3	0	0	0	11	3	0	0	3.44	5.24	1.31

	Kola																	Kola	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	3	1	0	5	6	0	9	0	0	3	0	0	3	0	0	0	1.88	2.75	0.69
Titan	0	1	0	0	0	0	4	0	0	5	2	0	0	0	0	0	0.75	1.57	0.39
Brock	4	0	2	0	0	0	0	0	0	0	1	0	0	0	1	0	0.50	1.10	0.27
Bransen	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0.06	0.25	0.06

	Wren																	Wren	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	0	14	2	2	1												3.80	5.76	2.58
Titan	2	11	7	0	1												4.20	4.66	2.08
Brock	0	4	0	1	0												1.00	1.73	0.77
Bransen	5	0	0	2	2												1.80	2.05	0.92

Oestrus sniff duration (seconds) followed by the mean, standard deviation and the standard error (continued).

	Tori																	Tori	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	0	6	14	0	5	12	4	0	0	0	2	4					3.92	4.80	1.45
Titan	10	0	0	0	5	0	0	4	12	6	0	4					3.36	4.23	1.28
Brock	0	0	0	3	1	6	0	0	0	0	6	2					1.50	2.32	0.70
Bransen	3	0	7	0	0	1	0	0	1	1	0	1					1.17	2.04	0.61

	Nui																	Nui	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	5	0	0	0	0	0	0										0.71	1.89	0.71
Titan	0	0	0	0	5	0	0										0.71	1.89	0.71
Brock	18	4	1	0	0	0	0										3.29	6.65	2.51
Bransen	1	0	2	0	1	1	1										0.86	0.69	0.26

	Chyna																	Chyna	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	12	0	0	0	0	0	0	4	9	0	0	0	0	0	2	0	1.69	3.65	0.91
Titan	0	4	0	7	0	0	29	4	0	0	5	0	0	0	0	4	3.31	7.25	1.81
Brock	14	0	0	5	6	0	0	4	0	3	0	0	0	4	0	6	2.63	3.86	0.97
Bransen	0	3	5	0	8	6	12	0	2	4	0	5	3	0	5	0	3.31	3.48	0.87

	Muffy																	Muffy	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	6	6	0	1	0	4	4	0	0	0	4	0					2.08	2.50	0.72
Titan	0	0	0	1	0	0	0	6	0	7	0	0					1.17	2.52	0.73
Brock	19	0	7	4	5	1	0	0	0	11	1	0					4.00	5.89	1.70
Bransen	0	0	0	1	0	0	0	2	5	4	3	2					1.42	1.78	0.51

	Broom																	Broom	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	3	0	13	0	0	23	2	6	13	16	0	0					6.33	7.92	2.29
Titan	3	9	1	0	1	0	0	0	5	0	2	0					1.75	2.77	0.80
Brock	10	0	6	9	0	5	0	0	4	0	0	0					2.83	3.83	1.11
Bransen	7	2	0	3	0	0	2	0	0	0	0	3					1.42	2.15	0.62

	Suede																	Suede	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	3	10	3	8	0	1											4.17	3.97	1.62
Titan	0	6	0	9	2	0											2.83	3.82	1.56
Brock	13	8	0	0	0	8											4.83	5.60	2.29
Bransen	15	15	0	0	0	0											5.00	7.75	3.16

	Astra																		Astra	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	mean	s.d.	s.e.
Sunny	12	7	0	0	8	15	0	5	4	0	15	5	0	0	5	1	0	4.81	5.30	1.29
Titan	6	7	0	0	9	0	3	6	0	1	0	0	0	0	0	3	1	2.19	3.02	0.73
Brock	42	0	14	7	11	0	1	0	0	3	9	0	3	0	0	4	8	5.88	10.3	2.50
Bransen	12	17	9	11	0	5	8	0	0	0	0	0	0	4	0	1	0	4.19	5.46	1.32

Oestrus sniff duration (seconds) followed by the mean, standard deviation and the standard error (continued).

	Pippe																		Pippe	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	mean	s.d.	s.e.
Sunny	7	7	1	2	10	0	0	6	5	14	2	0	0	17	12	17	3	6.25	5.97	1.45
Titan	6	5	6	62	5	6	0	5	2	6	7	5	14	6	0	0	2	8.44	14.3	3.47
Brock	15	0	4	0	0	5	0	18	8	4	11	3	5	0	3	5	0	5.06	5.44	1.32
Bransen	2	2	9	0	0	0	2	1	1	2	17	0	0	2	0	2	4	2.50	4.32	1.05

	Ziggy																	Ziggy	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	19	18	9	0	0	3	10	0	1	24	0	2	0	0			6.14	8.45	2.26
Titan	0	0	0	0	4	6	11	5	5	0	3	0	0	0			2.43	3.39	0.91
Brock	11	0	0	8	0	0	0	6	5	9	0	0	8	0			3.36	4.24	1.13
Bransen	2	5	0	1	0	0	0	0	0	0	4	3	0	17			2.29	4.56	1.22

	Asia																	Asia	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	2	11	6	0	0	0	4	1									3.00	3.89	1.38
Titan	24	5	0	0	12	4	0	1									5.75	8.43	2.98
Brock	1	1	0	11	0	0	10	0									2.88	4.73	1.67
Bransen	2	0	0	1	0	4	5	4									2.00	2.07	0.73

	Ngaio																	Ngaio	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	4	7	0	0	0	0	16	0	6	4	0	2	0	6	4	0	3.06	4.31	1.08
Titan	12	0	0	0	5	0	0	0	8	0	0	2	4	0	0	0	1.94	3.59	0.90
Brock	35	5	3	0	0	5	7	0	0	0	4	0	6	0	0	0	4.06	8.64	2.16
Bransen	1	0	0	3	0	2	0	0	0	0	6	1	0	1	0	3	1.06	1.69	0.42

Appendix 3 continued. Number of oestrus visits followed by the mean, standard deviation and the standard error.

	Dusky																	Dusky	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	2	1	2	3	1	1	1	1	1	1	1	2	0	1	2	1	1.31	0.70	0.18
Titan	1	2	1	1	0	1	1	0	1	1	1	1	1	1	1	2	1	0.52	0.13
Brock	1	0	1	2	1	1	1	0	2	2	1	0	1	0	1	2	1	0.73	0.18
Bransen	0	1	2	1	1	1	0	2	1	0	0	2	1	1	1	1	0.94	0.68	0.17

	Fya																		Fya	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	mean	s.d.	s.e.
Sunny	0	1	1	1	0	0	1	1	0	0	0	1	0	1	0	1	0	0.47	0.51	0.12
Titan	1	2	1	0	1	3	1	1	1	1	0	0	0	0	2	1	1	0.94	0.83	0.20
Brock	0	1	0	1	1	1	1	0	0	2	1	0	0	1	1	1	0	0.65	0.61	0.15
Bransen	1	0	0	0	1	2	0	0	1	0	0	0	1	0	0	0	0	0.35	0.61	0.15

	Sheba																	Sheba	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	2	1	3	2	2	1	1	3	0	1	1	1	0	1	2	1	1.38	0.89	0.22
Titan	2	2	3	0	1	1	1	1	1	0	0	0	2	1	2	1	1.13	0.89	0.22
Brock	1	0	1	0	3	1	0	1	2	1	3	0	1	1	1	0	1	0.97	0.24
Bransen	1	1	2	0	1	1	1	1	2	2	1	1	0	1	1	1	1.06	0.57	0.14

	Bella																	Bella	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	0	0	1	2	0	0	2	0	0	0	2	1	1	0	0	0	0.56	0.81	0.20
Titan	0	3	2	0	1	1	1	2	1	1	1	0	2	0	0	0	0.94	0.93	0.23
Brock	1	0	0	0	1	1	2	1	1	0	1	2	2	1	0	1	0.88	0.72	0.18
Bransen	1	0	1	0	0	1	3	1	1	0	1	2	0	1	0	1	0.81	0.83	0.21

	Nikki																	Nikki	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	2	0	2	3	2	1	2	2	0	2	1	0	1	0	1	1	1.25	0.93	0.23
Titan	1	1	1	0	1	1	1	1	1	2	0	0	0	0	1	0	0.69	0.60	0.15
Brock	0	1	2	1	1	2	1	1	1	0	2	1	1	0	0	0	0.88	0.72	0.18
Bransen	0	1	0	0	1	0	2	2	1	0	0	0	2	1	0	0	0.63	0.81	0.20

	Kola																	Kola	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	1	1	0	1	1	0	1	0	0	1	0	0	1	0	0	0	0.44	0.51	0.13
Titan	0	1	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0.25	0.45	0.11
Brock	1	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0.25	0.45	0.11
Bransen	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0.06	0.25	0.06

	Wren																	Wren	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	0	2	1	2	1												1.2	0.84	0.37
Titan	1	1	1	0	1												0.8	0.45	0.20
Brock	0	1	0	1	0												0.4	0.55	0.24
Bransen	1	0	0	1	1												0.6	0.55	0.24

Number of oestrus visits followed by the mean, standard deviation and the standard error (continued).

	Tori																	Tori	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	0	1	1	0	2	2	1	0	0	0	0	1					0.67	0.78	0.23
Titan	2	0	0	0	1	0	0	2	1	1	0	0					0.58	0.79	0.24
Brock	1	0	0	1	1	2	0	0	0	0	1	1					0.58	0.67	0.20
Bransen	0	0	1	0	0	1	0	0	1	1	1	0					0.42	0.51	0.16

	Nui																	Nui	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Nui	s.d.	s.e.
Sunny	1	0	0	0	0	0	0										0.14	0.38	0.14
Titan	0	0	0	0	1	0	0										0.14	0.38	0.14
Brock	1	1	1	0	0	0	0										0.43	0.53	0.20
Bransen	1	0	1	0	1	1	1										0.71	0.49	0.18

	Chyna																	Chyna	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Chyna	s.d.	s.e.
Sunny	2	0	0	0	0	0	0	1	2	0	0	0	0	0	1	0	0.38	0.72	0.18
Titan	0	1	0	2	0	0	2	1	0	0	1	0	0	0	0	1	0.5	0.73	0.18
Brock	1	0	0	1	4	0	1	1	0	1	0	0	0	1	0	1	0.69	1.01	0.25
Bransen	0	1	1	0	1	1	0	0	1	1	0	1	1	0	1	0	0.56	0.51	0.13

	Muffy																	Muffy	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Muffy	s.d.	s.e.
Sunny	1	2	0	1	0	1	1	0	0	0	1	0					0.58	0.67	0.19
Titan	0	0	0	1	0	0	0	2	0	2	0	0					0.42	0.79	0.23
Brock	2	0	1	1	2	1	0	0	0	1	1	0					0.75	0.75	0.22
Bransen	0	0	0	1	0	0	0	1	1	1	0	1					0.42	0.51	0.15

	Broom																	Broom	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Broom	s.d.	s.e.
Sunny	1	0	3	0	0	3	1	1	1	2	0	0					1	1.13	0.33
Titan	1	3	1	0	1	0	0	0	1	0	1	0					0.67	0.89	0.26
Brock	2	0	1	1	0	1	0	0	1	0	0	0					0.5	0.67	0.19
Bransen	3	1	0	1	0	0	1	0	1	0	0	2					0.75	0.97	0.28

	Suede																	Suede	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Suede	s.d.	s.e.
Sunny	1	1	1	1	0	1											0.83	0.41	0.17
Titan	0	1	0	2	1	0											0.67	0.82	0.33
Brock	2	1	0	0	0	1											0.67	0.82	0.33
Bransen	2	1	0	0	0	0											0.5	0.84	0.34

	Astra																		Astra	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Astra	s.d.	s.e.
Sunny	1	1	0	0	1	2	0	1	1	0	1	1	0	0	1	1	0	0.65	0.61	0.15
Titan	1	1	0	0	2	0	1	1	0	1	0	0	0	0	0	1	1	0.53	0.62	0.15
Brock	2	0	1	1	1	0	1	0	0	1	1	0	1	1	0	1	1	0.71	0.59	0.14
Bransen	2	1	3	2	0	1	2	0	0	0	0	0	0	0	0	1	0	0.71	0.99	0.24

Number of oestrus visits followed by the mean, standard deviation and the standard error (continued).

	Pippe																	Pippe		
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	mean	s.d.	s.e.
Sunny	1	1	1	1	3	0	0	1	1	2	1	0	0	2	2	2	1	1.12	0.86	0.21
Titan	1	1	2	1	1	1	0	1	1	1	1	1	2	1	0	0	1	0.94	0.56	0.13
Brock	1	0	1	0	0	1	0	1	1	1	1	1	1	0	1	1	0	0.65	0.49	0.12
Bransen	1	2	1	0	0	0	1	1	1	1	1	0	0	1	0	2	2	0.82	0.73	0.18

	Ziggy																Ziggy		
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Ziggy	s.d.	s.e.
Sunny	3	3	2	0	0	1	2	0	1	2	0	1	0	0			1.07	1.14	0.30
Titan	0	0	0	0	1	1	1	1	1	0	1	0	0	0			0.43	0.51	0.14
Brock	1	0	0	1	0	0	0	1	1	1	0	0	1	0			0.43	0.51	0.14
Bransen	1	1	0	1	0	0	0	0	0	0	1	1	0	2			0.5	0.65	0.17

	Asia																Asia		
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Asia	s.d.	s.e.
Sunny	1	1	1	0	0	0	2	1									0.75	0.71	0.25
Titan	3	1	0	0	2	1	1	1									1.13	0.99	0.35
Brock	1	1	0	1	0	0	2	0									0.63	0.74	0.26
Bransen	1	0	0	1	0	1	2	1									0.75	0.71	0.25

	Ngaio																Ngaio		
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Ngaio	s.d.	s.e.
Sunny	1	1	0	0	0	0	1	0	1	1	0	1	0	1	1	0	0.5	0.52	0.13
Titan	1	0	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0.31	0.48	0.12
Brock	2	1	1	0	0	1	1	0	0	0	1	0	1	0	0	0	0.5	0.63	0.16
Bransen	1	0	0	1	0	1	0	0	0	0	1	1	0	1	0	1	0.44	0.51	0.13

Appendix 3 continued. Oestrus latency to first approach followed by the mean, standard deviation and the standard error. Dashes show a failure to approach the urine sample.

	Dusky																	Dusky	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	13	1	4	1	86	1	129	51	79	17	157	21	-	9	15	8	39.47	50.3	12.6
Titan	7	10	28	16	-	86	15	-	10	111	87	152	19	92	24	71	52	46.8	11.7
Brock	30	-	20	41	10	90	31	-	68	7	160	-	120	-	10	18	50.42	49.3	12.3
Bransen	-	33	6	144	99	82	-	1	1	-	-	34	26	1	33	177	53.08	59.4	14.9

	Fya																		Fya	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	mean	s.d.	s.e.
Sunny	-	72	4	2	-	-	9	10	-	-	-	28	-	1	-	22	-	18.5	23.7	5.7
Titan	13	6	17	-	13	12	94	14	9	15	-	-	-	-	5	27	6	19.25	24.3	5.9
Brock	-	19	-	7	10	38	29	-	15	7	10	-	-	25	70	112	-	31.09	32.6	7.9
Bransen	20	-	-	-	5	19	-	-	-	-	-	-	9	-	-	-	-	13.25	7.4	1.8

	Sheba																	Sheba	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	1	58	9	1	1	137	49	11	-	5	127	5	-	18	84	1	36.21	48.0	12.0
Titan	49	80	4	-	97	21	16	6	42	-	-	-	29	16	108	43	42.58	35.1	8.8
Brock	143	-	66	-	23	11	-	175	3	99	6	-	11	39	174	-	68.18	68.3	17.1
Bransen	34	8	34	-	10	1	40	119	1	1	2	18	-	115	72	8	33.07	40.9	10.2

	Bella																	Bella	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	-	-	1	1	-	-	1	-	-	-	1	43	46	-	-	-	15.5	22.5	5.6
Titan	-	4	10	-	6	12	-	45	20	16	136	-	6	-	-	-	28.33	42.3	10.6
Brock	7	-	-	-	13	105	67	5	3	-	7	16	4	5	-	11	22.09	33.0	8.2
Bransen	7	-	33	-	-	21	6	2	5	-	116	2	-	1	-	1	19.4	35.5	8.9

	Nikki																	Nikki	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	9	-	28	22	8	28	39	14	-	63	75	-	9	-	11	27	27.75	21.7	5.4
Titan	72	72	22	-	142	16	106	118	21	53	-	-	-	-	19	-	64.1	45.9	11.5
Brock	-	97	75	51	104	6	59	110	37	-	73	94	65	-	-	-	70.09	31.3	7.8
Bransen	-	52	-	-	66	-	1	2	11	-	-	-	50	12	-	-	27.71	27.2	6.8

	Kola																	Kola	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	2	15	-	8	58	-	109	-	-	9	-	-	2	-	-	-	29	40.3	10.1
Titan	-	7	-	-	-	-	44	-	-	20	154	-	-	-	-	-	56.25	66.9	16.7
Brock	28	-	68	-	-	-	-	-	-	-	77	-	-	-	122	-	73.75	38.6	9.6
Bransen	-	-	-	-	-	18	-	-	-	-	-	-	-	-	-	-	18		

	Wren																	Wren	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	-	5	34	21	4												16	14.3	6.4
Titan	12	30	1	-	19												15.5	12.2	5.4
Brock	-	105	-	9	-												57	67.9	30.4
Bransen	6	-	-	65	1												24	35.6	15.9

Appendix 3 continued. Oestrus latency to first approach followed by the mean, standard deviation and the standard error (continued).

	Tori																	Tori	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	-	14	13	-	7	6	149	-	-	-	-	12					33.5	56.7	17.1
Titan	16	-	-	-	144	-	-	7	12	26	-	-					41	58.0	17.5
Brock	-	-	-	-	165	1	-	1	-	-	5	-					43	81.4	24.5
Bransen	3	-	28	12	-	171	-	-	28	53	4	4					37.88	56.5	17.0

	Nui																	Nui	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	-	-	-	143	-	-	-										143	#####	######
Titan	17	-	-	-	-	-	-										17	#####	######
Brock	-	-	-	12	5	66	-										27.67	33.4	12.6
Bransen	59	81	42	8	-	60	50										50	27.2	10.3

	Chyna																	Chyna	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	34	-	-	-	-	-	-	6	37	-	-	-	-	-	46	-	30.75	17.3	4.3
Titan	-	55	-	10	-	-	5	20	-	-	2	-	-	-	-	3	15.83	20.3	5.1
Brock	16	-	-	18	2	-	92	32	-	2	-	-	-	15	-	49	28.25	30.0	7.5
Bransen	-	1	4	-	80	8	-	-	4	14	-	45	68	-	7	-	25.67	30.5	7.6

	Muffy																	Muffy	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	29	1	-	47	-	20	1	-	-	-	4	-					17	18.6	5.4
Titan	-	-	-	44	-	-	-	1	-	5	-	-					16.67	23.8	6.9
Brock	1	-	3	5	5	18	-	-	-	26	123	-					25.86	43.8	12.6
Bransen	-	-	-	42	-	-	-	139	6	40	179	9					69.17	72.3	20.9

	Broom																	Broom	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	26	-	4	-	-	4	35	42	50	17	-	-					25.43	18.1	5.2
Titan	83	9	17	-	57	-	-	-	42	-	103	-					51.83	36.8	10.6
Brock	13	-	6	5	-	73	-	-	64	-	-	-					32.2	33.4	9.7
Bransen	4	112	-	130	-	-	3	-	-	-	-	1					50	65.1	18.8

	Suede																	Suede	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	71	133	127	40	-	20											78.2	50.7	20.7
Titan	-	144	-	31	25	-											66.67	67.0	27.4
Brock	69	109	-	-	-	10											62.67	49.8	20.3
Bransen	10	90	-	-	-	-											50	56.6	23.1

	Astra																		Astra	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	mean	s.d.	s.e.
Sunny	77	29	-	-	51	25	-	5	128	-	47	15	-	-	-	18	-	43.89	38.4	9.3
Titan	90	63	-	-	7	-	76	67	-	21	-	-	-	-	19	82	9	48.22	33.7	8.2
Brock	25	-	160	6	82	-	145	-	-	143	35	-	102	14	-	6	11	66.27	61.7	15.0
Bransen	14	11	3	28	-	12	1	-	-	-	-	-	-	-	-	165	-	33.43	58.7	14.2

Oestrus latency to first approach followed by the mean, standard deviation and the standard error (continued).

	Pippe																		Pippe	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	mean	s.d.	s.e.
Sunny	31	4	10	8	10	-	-	25	87	55	136	-	-	5	31	13	85	38.46	40.9	9.9
Titan	23	66	99	11	13	34	-	40	12	37	89	29	29	155	-	-	80	51.21	41.7	10.1
Brock	4	-	68	-	-	7	-	86	77	60	101	35	16	-	4	46	-	45.82	35.1	8.5
Bransen	66	17	12	-	-	-	9	83	67	66	8	-	-	29	-	29	5	35.55	29.1	7.1

	Ziggy																	Ziggy	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	20	5	5	-	-	1	7	-	17	3	-	63	-	-			15.13	20.5	5.5
Titan	-	-	-	-	47	45	122	32	1	-	10	-	-	-			42.83	43.0	11.5
Brock	41	-	-	22	-	-	-	20	29	147	-	-	26	-			47.5	49.3	13.2
Bransen	17	18	-	123	-	-	-	-	-	-	3	2	-	13			29.33	46.4	12.4

	Asia																	Asia	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	75	1	3	-	-	-	1	31									22.2	32.1	11.4
Titan	3	13	-	-	1	1	145	57									36.67	57.2	20.2
Brock	91	20	-	4	-	-	25	-									35	38.4	13.6
Bransen	33	-	-	61	-	68	16	21									39.8	23.5	8.3

	Ngaio																	Ngaio	
Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	mean	s.d.	s.e.
Sunny	168	2	-	-	-	-	18	-	1	1	-	1	-	3	81	-	34.38	60.6	15.1
Titan	2	-	-	-	13	-	-	-	40	-	-	4	1	-	-	-	12	16.4	4.1
Brock	17	11	17	-	-	14	1	-	-	-	1	-	39	-	-	-	14.29	12.8	3.2
Bransen	68	-	-	11	-	95	-	-	-	-	76	7	-	23	-	1	40.14	38.4	9.6

Appendix 4

Number of sniffs and visits for each animals in response to each of the scents presented followed by the mean, standard deviation and standard error.

	Cheetah Male		**Cheetah Male**		**Cheetah Male**		**Cheetah Male**	
SMELL	Mbili sniff	Mbili visits	Tatu sniff	Tatu visits	Moja sniff	Moja visits	Kaitaia sniff	Kaitaia visits
Urine	0	0	5	4	1	1	4	2
	3	1	4	1	3	3	0	0
	3	2	1	1	3	2	7	1
	3	2	3	1	3	1	6	2
Mean	2.25	1.25	3.25	1.75	2.5	1.75	4.25	1.25
sd	1.50	0.96	1.71	1.50	1.00	0.96	3.10	0.96
se	0.75	0.48	0.85	0.75	0.50	0.48	1.55	0.48
Feliway	0	0	4	1	1	1	8	4
	3	2	10	7	3	2	3	3
	3	2	5	3	2	1	6	6
	10	1	6	3	3	1	1	1
Mean	4	1.25	6.25	3.5	2.25	1.25	4.5	3.5
sd	4.24	0.96	2.63	2.52	0.96	0.50	3.11	2.08
se	2.12	0.48	1.31	1.26	0.48	0.25	1.55	1.04
Mint	2	2	5	2	2	1	1	1
	2	1	2	2	1	1	0	0
	1	1	4	2	0	0	2	2
	1	1	3	2	1	1	1	1
Mean	1.5	1.25	3.5	2	1	0.75	1	1
sd	0.58	0.50	1.29	0.00	0.82	0.50	0.82	0.82
se	0.29	0.25	0.65	0.00	0.41	0.25	0.41	0.41
Catnip	0	0	7	6	1	1	0	0
	2	1	5	1	1	1	0	0
	2	1	5	1	1	1	0	0
	2	2	4	3	2	2	0	0
Mean	1.5	1	5.25	2.75	1.25	1.25	0	0
sd	1.00	0.82	1.26	2.36	0.50	0.50	0.00	0.00
se	0.50	0.41	0.63	1.18	0.25	0.25	0.00	0.00
Mouse	0	0	2	1	0	0	1	1
	1	1	3	1	1	1	0	0
	2	1	3	2	3	2	4	1
	1	1	2	1	3	1	3	1
Mean	1	0.75	2.5	1.25	1.75	1	2	0.75
sd	0.82	0.50	0.58	0.50	1.50	0.82	1.83	0.50
se	0.41	0.25	0.29	0.25	0.75	0.41	0.91	0.25
Blank	0	0	0	0	0	0	0	0
	1	1	1	1	0	0	0	0
	1	1	2	2	0	0	0	0
	0	0	1	1	0	0	0	0
Mean	0.5	0.5	1	1	0	0	0	0
sd	0.58	0.58	0.82	0.82	0	0	0	0
se	0.29	0.29	0.41	0.41	0	0	0	0

Number of sniffs and visits for each animals in response to each of the scents presented followed by the mean, standard deviation and standard error.

	Cheetah Female		**Cheetah Female**	
SMELL	Kazkazi sniff	Kazkazi visits	Yatima sniff	Yatima visits
Urine	4	2	4	4
	6	2	9	4
	0	0	1	0
	7	2	5	1
Mean	4.25	1.5	4.75	2.25
sd	3.10	1.00	3.30	2.06
se	1.55	0.50	1.65	1.03
Feliway	25	5	26	4
	8	4	5	2
	11	6	14	12
	12	4	17	4
Mean	14	4.75	15.5	5.5
sd	7.53	0.96	8.66	4.43
se	3.76	0.48	4.33	2.22
Mint	0	0	0	0
	5	5	3	3
	3	3	3	3
	3	3	9	4
Mean	2.75	2.75	3.75	2.5
sd	2.06	2.06	3.77	1.73
se	1.03	1.03	1.89	0.87
Catnip	0	0	0	0
	1	1	3	3
	1	1	0	0
	2	2	2	2
Mean	1	1	1.25	1.25
sd	0.82	0.82	1.50	1.50
se	0.41	0.41	0.75	0.75
Mouse	2	2	3	3
	1	1	2	2
	3	1	6	2
	3	3	1	1
Mean	2.25	1.75	3	2
sd	0.96	0.96	2.16	0.82
se	0.48	0.48	1.08	0.41
Blank	1	1	2	2
	1	1	0	0
	1	1	3	3
	1	1	0	0
Mean	1	1	1.25	1.25
sd	0	0.00	1.50	1.50
se	0	0.00	0.75	0.75

Number of sniffs and visits for each animals in response to each of the scents presented followed by the mean, standard deviation and standard error.

	Serval Male		**Serval Female**		**Tiger Male**	
SMELL	Zulu sniff	Zulu visits	Sheba sniff	Sheba visits	Sam sniff	Sam visits
Urine	4	4	4	4	3	2
	7	7	2	2	4	2
	4	4	5	5	7	5
	0	0	12	12	4	2
Mean	3.75	3.75	5.75	5.75	4.5	2.75
sd	2.87	2.87	4.35	4.35	1.73	1.50
se	1.44	1.44	2.17	2.17	0.87	0.75
Feliway	1	1	20	5	2	1
	6	1	0	0	3	2
	5	4	18	5	3	2
	0	0	25	8	0	0
Mean	3	1.5	15.75	4.5	2	1.25
sd	2.94	1.73	10.90	3.32	1.41	0.96
se	1.47	0.87	5.45	1.66	0.71	0.48
Mint	4	4	5	5	4	4
	2	2	4	4	3	2
	0	0	5	5	1	1
	4	4	6	6	2	2
Mean	2.5	2.5	5	5	2.5	2.25
sd	1.91	1.91	0.82	0.82	1.29	1.26
se	0.96	0.96	0.41	0.41	0.65	0.63
Catnip	3	3	2	2	9	9
	0	0	3	3	2	2
	0	0	17	11	2	2
	0	0	5	5	0	0
Mean	0.75	0.75	6.75	5.25	3.25	3.25
sd	1.50	1.50	6.95	4.03	3.95	3.95
se	0.75	0.75	3.47	2.02	1.97	1.97
Mouse	8	5	10	4	8	5
	7	3	7	7	3	2
	8	4	8	6	1	1
	8	7	4	3	1	1
Mean	7.75	4.75	7.25	5	3.25	2.25
sd	0.50	1.71	2.50	1.83	3.30	1.89
se	0.25	0.85	1.25	0.91	1.65	0.95
Blank	0	0	1	1	1	1
	1	1	0	0	1	1
	1	1	1	1	0	0
	0	0	0	0	0	0
Mean	0.5	0.5	0.5	0.5	0.5	0.5
sd	0.58	0.58	0.58	0.58	0.58	0.58
se	0.29	0.29	0.29	0.29	0.29	0.29