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**CHEMOCOMMUNICATION AND SOCIAL BEHAVIOUR
IN THREE *PANTHERA* SPECIES IN CAPTIVITY,
WITH PARTICULAR REFERENCE
TO THE LION, *P. leo*.**

BY

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for the degree of Doctor of Philosophy.

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"We make for ourselves in truth, our own spiritual world monsters, chimeras, angles; we make objective what ferments in us. All is marvellous for the poet; all is divine for the saint; all is great for the hero; all is wretched, miserable, ugly and bad for the base and sordid soul. The bad man creates around him a pandemonium, the artist an Olympus, the elect soul a paradise, which each of them sees for himself alone. We are all visionaries, and what we see is our soul in things."

Amiel.

"Lucinda looked straight ahead. She was moved, of course she was moved. The fools had worked so hard to please her. But she was angry, too, and the tears that ran down her cheek were caused by quite different forces from those which were producing the identical phenomenon in her lodger. Both lots of tears were salt, I am sure, and were probably within the normal range of salinity, i.e. between one per cent and two per cent salt, but this is merely to show you the limits of chemistry, for while Lucinda's tears were produced by diametrically opposed emotions, Oscar's were all in one direction and had their source in such grand territories as joy, wonder, humility, and love for these suit-trussed workers who had publicly enacted love for him, a stranger and an outcast."

"Oscar and Lucinda", Peter Carey.

Preface

This thesis is the result of my own research, and contains no work done in collaboration except where otherwise stated. The text does not exceed 80,000 words. No part of this thesis has been submitted to any other university in application for a higher degree.

Kirsti F. Anderson

SUMMARY.

This project is a contribution towards the understanding of the mechanisms involved in the chemocommunication systems of large mammals. Data are included on the social behaviour and use of scent marking for the African lion, *P. leo* as well as two other *Panthera* species namely the Siberian tiger, *P. tigris altaica*, and the leopard, *P. pardus*. The study was conducted in three Zoos or Safariparks in Denmark: København Zoo, Givskud Safaripark and Knuthenborg Safaripark.

Data were collected in three ways. The first part of the project comprised behavioural observations of the undisturbed social and marking patterns of the study groups. Particular attention was paid towards *Spraymarking*, *Scrape/urination* and normal *Urination* but *Clawing*, *Chinrubbing* and *Defaecation* were also recorded. Methods were developed which made it possible to collect samples of scent marks from the study animals. The scent samples collected were used in the second part of the field work, which involved an experimental investigation in which the animals were presented with scent marks from foreign individuals of varying sex, age or reproductive status. These experiments were conducted both in the "natural" setting of the outdoor enclosures as well as in the artificial surroundings of the night cages of the animals. In the third and final part of the study the scent mark samples collected were subjected to chemical analysis using the "Headspace" procedure on a combined Gas Chromatograph and Mass Spectrometre.

The activity and social interactions of the animals, although under influence of the captive environment, were generally close to what one would expect to see for wild animals. Each animal showed a distinct activity pattern and social repertoire, but more general differences between the sex/age groups were also found. Males tended to stand more than females or cubs and show higher levels of *Investigation*, whereas females generally moved more than males. Cubs played more than the two adult groups. These trends were seen in all three species.

Each animal had a distinct marking repertoire, but generally the male patterns were dominated by *Spraymarking* followed by *Scrape/urination*, whereas the female patterns were much more variable. Male lions had higher rates of *Spraymarking* and significantly higher rates of *Scrape/urination* than females. No significant difference was found between *Spraymarking* rates of male and female tigers, but both had significantly higher rates than castrated tiger males. The leopard male had higher rates of the two marking

types than the female.

Male lions showed a bias towards *Spraymarking* over *Scrape/urination* in territorial contexts, but no such bias in social contexts. The females showed no bias in markings in either context.

Investigation of experimental marks were dominated by *Scenting* and *Flehmen* and very little *Licking* and *Overmarking* was seen.

In the chemical analysis 58 compounds were identified in lion urine samples. Lion male samples overlapped significantly more in compound composition with other male samples than they did with female samples. The majority of the lions overlapped more within their own samples than they did with samples from other lions, and for seven out of 16 lions this difference was significant. Male lions had a significantly higher absolute concentration of 2-butanone than females, and females had a significantly higher relative concentration of acetone than did males.

57 compounds were identified in tiger urine samples. All tigers overlapped more within their own samples than between theirs and other samples from same sex individuals, but only one significantly so. Castrated tiger males had higher concentration of 5-hydroxy-4-methyl-6-hepten-3-one than the intact male.

In leopard urine samples 18 compounds were identified.

Possible candidates for species and sex identifying compounds were put forward for lions and tigers. On average two lion samples overlapped significantly more in compound composition than did a lion sample and a tiger sample. This was also the case for the overlap between two tiger samples compared to a tiger and a lion sample. Leopard samples overlapped equally with lion and tiger samples.

The results are discussed in relation to what is known about the same species living in the wild. Furthermore the use of chemical signals are discussed in relation to their chemical and physical properties, and their function in a territorial context is commented upon.

ACKNOWLEDGEMENTS.

During my work on this project a number of people have lent a helping hand.

First of all I would like to thank my supervisor Dr S.K. Eltringham of the Department of Zoology, University of Cambridge. Throughout my work he has offered advice and guidance, and his red pen has danced many a dance across the pages of drafts of this thesis. Further thanks are due to Dr. John Flowerdew and Dr. Claus Peter Bjaelke Hansen for providing additional commentary on my drafts. Dr. Simon Laughlin has given me much encouragement and advised me in procedural matters.

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CHAPTER 1: INTRODUCTION.

The use of chemical compounds in the transfer of signals and messages is an almost universal phenomenon in living organisms. It is seen on both an intra- as well as an intercellular level in plants, insects, fish and all the way up to the large mammals. At a cellular level this process is often very straightforward with a chemical compound being released from, say, one end of a synapse. It then diffuses across the space between the neurones and is bound to specific receptors on the other side. This binding can result in a depolarisation of the receptor cell creating an action-potential which propagates the signal/message throughout the relevant part of the nervous system. This microprocess also forms the basis for the more complex macroprocess, which is seen in the inter-individual communication in the larger mammals. Here, usually a whole cocktail of chemical compounds is released at the same time, and the relative concentrations of individual constituents may be significant for the meaning of the message transferred. The target of a given scent mark, rather than being a specific individual may be a potential intruder, a potential mate, another group member or an individual in some other such general category. Consequently the study of this type of communication becomes a highly complex task.

A closer investigation of the chemical compounds used in scent communication by animals only complicates matters further. The aroma intensity or threshold value of different compounds varies enormously e.g. in humans the threshold value for detecting ethanol (in μg per litre of water) is 100,000 whereas for β -ionone it is as low as 0.007 (MacLeod 1980). This means that compounds with a low threshold value, even at very small concentrations, may have a significant value in communication. Further complications follow from the fact that compounds of similar chemical structure may have very different aromatic and odour qualities. Even for structurally similar compounds, which also have similar odour qualities, the intensities may vary considerably. There are also examples of chemicals of different structure that possess very similar odour qualities. Furthermore, synergistic effects are found in which two or more chemicals, each at a sub-threshold level, together form a mixture with a perceptible odour.

Scent marks are deposited on a variety of surfaces, depending on species, habitat and the availability of scent posts. The nature of the surface on which the mark is deposited has an effect on the rate of release of the active components. As a scent mark is usually a cocktail of different compounds that are deposited at the same time, the quantitative and

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qualitative nature of the inactive constituents (such as lipids) will have an influence on the evaporation rate of the active part(s). Furthermore the release may be modulated by other environmental conditions, such as humidity or wind speed, and the degree of modulation will depend on the relative exposure of the mark to these factors (Regnier and Goodwin 1976; Alberts 1992).

The actual communicatory functions of marking with odours are as yet poorly understood, but several hypotheses have been put forward as to what the functions might be. One of the earliest recognised functions of scent marking is its use in territorial behaviour by many mammals (Hediger 1949) but the exact way in which it works is still not known. Hediger (1949) suggested that it acts by deterring potential intruders from entering the marked area. Another possible explanation was given by Geist (1964), who speculated that scent marks may intimidate an intruding animal, so that once it has entered the territory of another animal it loses confidence and becomes more ready to flee. It has also been suggested that the territory holder may increase its own confidence by repeated marking of scent posts, whereby it also familiarises itself with the territory and increases its level of orientation within its borders (Hediger 1949; Walther 1978; von Richter 1972). Taking these ideas one step further Gosling (1982, 1990) speculated that the intruder, by learning the scent of the resident male from the scent posts it encounters, is able to identify him as such by matching the territorial scent to the scent of the animal, and once this identification is made, the intruder, unless he wants to contest the ownership of the territory, is able to take appropriate evasive action and thus avoid the risk of serious injuries. A basic assumption for all these hypothesis is that scent marks contain, and are able to pass on, information about identity and status of individuals and the use (spatial information) and frequency of use (temporal information) of space as home-ranges or territories. In addition scent marking may attract and/or stimulate potential mates (Noble 1939, Kleiman 1966, Johnson 1973), but this function may be limited to specific breeding seasons or oestrous periods. A further and probably unintentional communicatory function of scent marks is the possible ability of predators and prey species to recognise the scent of each other and thereby promote hunting behaviour in the predators and increased vigilance or avoidance in the prey species. All the different hypotheses are attempts to explain why a given animal marks its surroundings with its own scent, and also what information these scent marks may possibly convey. This, however, is only one side of the story. Another side, which has to be taken into account when discussing communication by scent marking, is that although a given scent mark may contain a variety of information, the animal which investigates the scent mark may only react to the information which is

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relevant to it in the given situation and ignore the rest. Therefore two animals encountering the same scent mark may react to it in very different ways depending on their sex, age, previous experiences and present motivations.

Scent communication in mammals.

The scent communication of mammals has been studied by numerous workers over the last 50 years, but it is only within the last 20 or so years that chemical analyses have become incorporated into these studies on a routine basis. The literature on scent communication in mammals has been reviewed by Bossert and Wilson 1963; Eisenberg and Kleiman 1972; Johnson 1973; Thiessen and Rice 1976; Wemmer and Scow 1977; Brown 1979; Macdonald 1980; Halpin 1980, 1986; Gosling 1982; Albone 1984; Jannett 1984 as well as Brown and Macdonald 1985. In the following pages a number of specific studies will be mentioned in order to cast light on the advances made within this field.

Rodents and Lagomorphs:

Several studies have examined the diverse use and possible function of social odours in a variety of species, the majority being small rodents. The scent marking of mice (*Mus musculus* and *Mus domesticus*) has been studied extensively. Wolff and Powell (1984) investigated counter-marking in mice by presenting different types of urine marks to males and females. They found that intact male mice marked more when presented with pre-oestrous and ovariectomised urine marks from females than when presented with oestrous marks. Castrated males did very little marking in response to experimental marks. Oestrous females marked more than non-oestrous females in response to male marks. Wolff and Powell concluded that both the chemical composition as well as the pattern of urine marking could convey information about reproductive status. Sandnabba (1986) looked into the effects of aggression on scent marking in male mice. Those repeatedly defeated in fights urinated in larger amounts and in fewer spots than control males. Males of a different control strain preferred areas marked with urine from the defeated male mice rather than from the control male mice, whereas females showed the opposite preference. He therefore concluded that individual levels of aggressiveness influenced the composition and pattern of scent marking. Bishop and Chevins (1987) also found that subordinate

males mark in fewer areas and make larger spots than dominant ones, and that these spots tended to be at the periphery of the test area, whereas the dominant males marked evenly throughout the area. Test mice avoided areas marked with urine from dominant territory-holding males. Their conclusion was similar to that of Sandnabba, namely that the urine of dominant territorial males contains a factor which deters subordinates from entering the marked area.

Hurst (1987, 1989, 1990a,b,c,d) carried out a number of studies on a population of wild house mice (*Mus domesticus*). In her first study (Hurst 1987), she found that urine smearing was used in providing cues for orientation and to help in the detection of novel objects. In 1989 she conducted a series of odour tests and found that there was a lack of investigation and a low marking response of adult males towards unfamiliar male urine, that male and female mice of all ages, except breeding females, showed prolonged investigation of female urine marks, and that subadult males showed intense investigation of adult male urine. She concluded that urine marks contain information on dominance and reproductive status. In her 1990 studies she found that dominant males strongly counter-mark resident and non-resident male marks, and also that subordinate males closely investigated the marks of dominant males (Hurst 1990a). She concluded that urine marks left by dominant males were used by subordinate males to orient themselves within the territory in which they resided and away from areas marked by other dominant males. For females, she found that breeding resident females strongly counter-marked marks of neighbouring breeding females (Hurst 1990b). She concluded that females mark at high frequencies to advertise their dominant breeding status to other females. She also found that dominant males counter-marked all types of female urine at high rates (Hurst 1990c). In a later article Hurst and co-workers found that males in a resident and intruder situation exhibited more aggression than when two males were put together in a clean enclosure. They concluded that this result was more consistent with competition for dominance over suitable sites than investment in fierce aggression to drive competitors away (Hurst *et al.* 1996).

Coquelin (1992) found that females marked most frequently in the presence of a male stimulus, and that their marking rate was not influenced by the presence of stimuli from other females. The marking rate of the females remained relatively steady throughout gestation but declined dramatically at parturition although it gradually increased again to the pre-mating level. He therefore concluded that female urine marks served to evoke endocrine and behavioural responses in proximate males rather than to attract distant males. Palanza *et al.* (1994) observed that female mice, although not aggressive to other

females in the absence of males, became aggressive and territorial when exposed to a dominant territory-holding male. They concluded, therefore, that females compete for access to a territory-holding male, and that scent marking is part of this competition. Mossman and Drickamer (1996) observed that oestrous females preferred odours from dominant rather than subordinate males whereas nonoestrous females showed no significant preference. Furthermore, Drickamer (1997) found that dominant males were more attracted to odours from oestrous females than to odours from nonestrous females or pregnant/lactating females. Gosling and McKay (1990) demonstrated that the functional aspects of the hypothesis of scent-matching may be in operation in mouse conflict behaviour.

It has been shown that mice have the ability to recognise and remember the scent of a particular conspecific. If pregnant female mice are exposed to the pheromones of male mice, those originating from males other than her mate can result in pregnancy loss, whereas those originating from her mate does not have this effect (Bruce 1960).

The chemical composition of mouse urine was studied by a group of scientists working at Indiana University, USA (Novotny *et al.* 1985, 1990; Jemiolo *et al.* 1985, 1986, 1992; Schwende *et al.* 1986). They found 61 different compounds in samples of male and female urine. Two sulphur compounds (3,4-dehydro-*exo*-brevicomine and *p*-toluidine) were specific to the male urine and behavioural tests showed that they were important in male-female communication. Dominant males produced higher quantities of sesquiterpenes than subordinate males, and the presence of these compounds was shown to repress the marking behaviour of subordinate males. The castration of males lead to a decrease in concentration and number of urine compounds. Urine of oestrous females contained a specific compound (*n*-pentyl acetate) and had general higher concentrations of others. Species specific compounds were also identified.

Another species of small rodent which has been studied extensively is the Mongolian gerbil (*Meriones unguiculatus*). Probst and Lorenz (1987) found that the part of the female gerbil urine which could stimulate scent marking in males was contained in the hydrophilic non-volatile fraction. In a later study Probst and Schonheiter (1993) showed that the stimulation of male scent marking was dependent on the reproductive state of the female, with only urine from pre-oestrous animals being able to stimulate the males. On this evidence they concluded that scent marking plays an important role in the sexual behaviour of the gerbils.

In the bank vole (*Clethrionomys glareolus*), Brinck and Hoffmeyer (1984) found that

marking urine contained a compound from the preputial gland that was not found in metabolic urine. They identified this compound as hexadecyl acetate. They also found that females responded more strongly to male preputial gland secretion and male marking urine than to metabolic urine. Also hexadecyl acetate-enriched urine provoked a stronger response in dominant males than urine alone, and the opposite effect was found for subordinate males. On this basis the authors suggested that hexadecyl acetate has a function in the voles' dominance interactions. Johnston et al. (1997) found that female meadow voles (*Microtus pennsylvanicus*) were able to distinguish between and indeed preferred the most recently deposited scent mark of two marks placed on top of each other. They concluded that male counter-marking may be competitive advertising which females could use in mate choice decisions.

Mykytowycz and co-workers have extensively studied the marking behaviour and glands of the rabbit (*Oryctolagus cuniculus*). The chin gland of this animal plays an important role in the territorial system of rabbits, and the secretory activity of the gland is positively correlated with social rank, confidence of territory holder, testosterone levels and reproductive activity (Mykytowycz 1962, 1965; Mykytowycz and Dudzinski 1966; Mykytowycz et al. 1976; Bell 1985; Hudson and Vodermyer 1992). Urine marks were less effective at evoking responses in rabbits than secretions from the chin glands.

Two different species of hamsters have been investigated. One was the solitary golden hamster (*Mesocricetus auratus*) and the other the more gregarious dwarf hamster (*Phodopus sungorus campbelli*). In both species male hamsters increased their marking rates when presented with marks from other males and non-oestrous females (Johnston 1975; Reasner and Johnston 1987). In the dwarf hamster it was also noted that when males investigated scent marks from other males their attention was focused on the mark itself, but while they were investigating female marks, their attention, after a short investigation of the mark itself, was directed to the surrounding area (Reasner and Johnston 1987). In the solitary golden hamster Johnston et al. (1993) showed by a series of habituation experiments that males could discriminate between individuals on the basis of scent samples from the flank glands, urine and faeces. Steel and Keverne (1985) found that intact males, after having been presented with a sample of vaginal odour, could discriminate between the donor and non-donor females. Petrulis and Johnston (1997) found that some odours containing classes of information, such as sexual identity, did not always influence scent marking behaviour, and they concluded that more specific effects of particular odours, rather than general classes, were involved in marking responses. In a

more recent study Johnston *et al.* (1994, 1995) found that when two marks were presented one on top of the other (scent overmarking), the hamsters would remember only the scent of the top (fresh) mark, thus indicating a mechanism of scent masking. In another study Johnston and Robinson (1993) showed that the two species of hamsters reacted in a similar way when presented with flank gland secretion from the first species and ventral gland scent from the second, thus demonstrating a lack of species-specificity in the scent marks.

Carnivores:

Moving on to the carnivores, three species which have been well studied are the coyote (*Canis latrans*), the wolf (*Canis lupus*) and the red fox (*Vulpes vulpes*). The first two of these species are territorial and group living with a dominant male and female in charge of the pack, whereas the fox although also territorial, lives in groups of only one adult male and one or more related adult vixens. For the coyote Bowen and McTaggart Cowan (1980) distinguished between six types of scent marking. These were urine alone, urine with scratching (ground scratching with hind paws), urine and faeces with scratching, faeces alone, urine and faeces, and faeces with scratching. The most common of these marking types was urine alone (66%) followed by urine with scratching (29%). A higher density of scent marks was found along the borders of the territory than towards the centre. This was found for all mark types. Dominant pack members marked more than subordinates, and the dominant male marked more than the dominant female. Border marks from neighbouring packs were overmarked by the resident pack. Barrette and Messier (1980) found similar proportions of urine marking (55%) and urine plus scratching (25%) in their study of coyotes. Gese and Ruff (1997) reported 86% urinations and only 14% scratchings for their study pride in Yellowstone National Park. The alpha pair were the most active scent markers and their rate of mark deposition increased during the breeding season whereas those of beta and cub coyotes varied little throughout the year. The territorial boundaries received more scent marks than expected compared to the interior. Transient or nomadic individuals scent marked less than did the resident packs. They also found that the rate of scent marking by individuals in a resident pack was independent of pack size.

The scent marking behaviour of the wolf is quite similar to that of the coyote. Raised Leg Urination (RLU) is considered the most important form of scent communication in the wolf, making up 60-80% of all scent marks observed (Peters and Mech 1975). As for

the coyotes a higher density of scent marks was found along the borders of the territory than in the centre. Again it was mostly the dominant pair which performed the scent marking. Marking rates increased when wolves encountered border marks from neighbouring packs. Rothman and Mech (1979) concluded that wolves which RLU marked were generally established breeders, whereas wolves which did not show RLU did not breed. They also suggested that lone nomadic wolves use scent marks to navigate between territories, and to locate an unoccupied area where they can establish themselves. Raymer *et al.* (1984, 1986) identified 77 different compounds in the bladder urine of wolves. Castration of male wolves led to a decline in the concentration of most compounds as well as in the actual number of compounds. Asa *et al.* (1990) found that serum testosterone levels were positively correlated with urine marking rates in dominant wolves of both sexes. Asa *et al.* (1985) who studied captive wolves and Vila *et al.* (1994) who studied wild wolves both found that faeces were left in a non-random manner and suggested that faeces also play a role in the scent marking behaviour of the wolf.

In the red fox the most common form of scent marking has been termed token urination. The token urinations, in which the animal sprinkles a few drops of urine, are distinguished from normal squat urinations by their brevity and the choice of urination site. Token marking was almost exclusively confined to within the territorial boundaries of a given group. There appeared to be a social depression of token marking in subordinate vixens. After inter- and intra-group conflicts the victorious animal would show an increased rate of token marking for a short period (Macdonald 1979). Goszczynski (1990) found no difference in the intensity of scent marking between the centre and the borders of fox territories in Poland. The red fox uses token urination to optimise its feeding behaviour by marking places where it has already eaten but where food odours or inedible pieces remain (Henry 1977). The chemical composition of fox urine was first studied by Jorgenson *et al.* (1978). The urine was collected from snow on which the foxes had marked. They identified a range of different compounds including four which appeared to be species-specific for the fox. Another compound, 2-methylquinoline, was found in male urine only. Wilson *et al.* (1980) tested a solution made from synthetic copies of eight of the compounds identified by Jorgenson *et al.* (1978) against a control solution. They found that the test solution provoked significantly more counter marking by wild foxes than did the control solution (also reported in Whitten *et al.* 1980). Bailey *et al.* (1980) studied the volatile part of fox urine and found a possible seasonal fluctuation for some of the compounds identified. In contrast to Jorgenson *et al.* (1978) they also found 2-

methylquinoline in the urine of female foxes.

The scent marking behaviour of the brown hyaena (*Hyaena brunnea*) was studied by Mills *et al.* (1980). They found that the brown hyaenas in the Kalahari lived in small groups on territories up to 550 km² in size, and that this species deposited two different types of paste, white and black, from the anal pouch on stalks of grass around the group territory. The density of scent marks was positively correlated with the time that the hyaenas spent in a given area, but the frequency of pastings increased when the hyaenas came near to the territorial boundary. Chemical analysis of the two scent mark types showed a distinct chemical composition for each, and the concentration of the major compounds showed distinct differences between two individuals but only minor variation within each individual. The authors suggested that the function of pasting is to inform the group members of each others' movements, as well as to tell non-resident hyaenas that the territory is occupied. Kruuk (1972) found a different scent marking pattern for the spotted hyaenas (*Crocuta crocuta*) in the Ngorongoro Crater, which live in large social groups (30-80 individuals) on relatively small territories (ca. 30 km²). These territories are marked predominantly along the boundaries on regular border patrols. In a later study on the same species, Mills and Gorman (1987) found that the Kalahari population of spotted hyaenas, which live in smaller groups on larger territories than their Ngorongoro counterparts, have a scent marking pattern similar to that seen in the brown hyaena living in the same habitat. They therefore concluded that the pattern found by Kruuk, rather than being a species-specific difference in marking pattern between the two hyaena species, was a consequence of the difference in group size and territory size between the two habitats. They argued that it would be impossible for members of a small group living on a large territory to keep up a high level of scent marking along the entire border, and that they would be more likely to mark the core areas of the territory intensely and the borders on a less regular basis. Woodmansee *et al.* (1991), in a study of a group of captive spotted hyaenas, found that the rate of pasting behaviour increased for young animals as they approached sexual maturity. In addition they found that gonadectomy during the early juvenile age period had no significant influence on the subsequent pasting frequency. Buglass *et al.* (1990) analysed the anal sac secretion of three different hyaenas namely the brown, the striped (*H. hyaena*) and the spotted hyaena. They identified 40 different compounds (hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and sulphur compounds) 17 of which were found in the brown, 19 in the striped and 25 in the spotted hyaena. The overlap in compound composition between the brown and the striped hyaena

was 67%, between the brown and the spotted hyaena 35% and between the striped and the spotted hyaena 32%.

Another species within the Hyaenidae which uses pasting of anal sac secretion in its territorial marking is the aardwolf (*Proteles cristatus*). This solitary species is smaller than the hyaenas and is entirely insectivorous (Nel and Bothma 1983). Apps *et al.* (1989) analysed the scent marks and anal sac secretion of the aardwolf and found that both consisted mostly of short- to medium-chain fatty acids and a complex series of medium- and long-chain esters of the fatty acids. The scent marks also contained indole and hexanol. They found differences between the individuals in the compound composition and relative concentration of peaks. The odour of the anal gland secretion was detectable by the human nose for up to six months. Richardson (1990, 1991, 1993) found that aardwolves concentrate scent marks along territorial borders and that the density of scent marks increases as the territorial size decreases. Middens are also thought to play a role in territorial marking. On the basis of his aardwolf data Richardson argued in favour of the intimidation function of scent marks. He based his argument on observations of the reaction of two adult males that were simultaneously trespassing on a third male's territory. When the two males became aware of each other they both fled towards their own territory without trying to establish each other's identity first. He therefore pointed out that in contrast to the ungulates, which Gosling (1982) used to illustrate his hypothesis of scent matching and ritualised conflict, no scent matching rituals can be found in carnivore species and the risk to an intruder, rather than being a ritualised conflict, is one of serious bodily injury or death.

The review presented above represents only a small part of the studies which have looked into scent marking behaviour and its functions in non-felid mammals. An exhaustive listing is beyond the scope of this thesis, but these studies serve the purpose of illustrating that scent marking is used in a variety of contexts and that it has a function in communication within and between sex and age groups. The following paragraphs will review the most relevant literature on scent marking in the felids.

Felids.

Most studies dealing with the marking behaviour of felids have been purely descriptive. One exception is the domestic cat (*Felis catus*), which has been studied quite extensively. The domestic cat shows a distinct flexibility when it comes to social organisation. In areas of low density they live a mostly solitary territorial existence, but in

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areas of high density they are able to live in social groupings (Jones 1977; Liberg 1980; Izawa *et al.* 1982; Macdonald *et al.* 1987; Liberg and Sandell 1988; Kerby and Macdonald 1988). Liberg (1980) found non-overlapping home ranges for the females of his study population in Sweden, each home range being inhabited by a group of females. The males were solitary with home ranges overlapping those of one or more female groups. Macdonald *et al.* (1987) also found that male cats had larger territories than females in English farm populations. The tom-cats marked their surroundings by spraying urine backwards while standing with the tail in a vertical position. Spraymarking has also been documented in females but at much lower rates than for the males (Feldman 1994). Further ways of marking are scratching trees, rubbing of cheeks against objects and depositing faeces. Differences in percentage distribution of marking types were found in adult males (Feldman 1994). It has been shown in laboratory experiments that tom-cats react in the same way to urine spray marks as to marks of normal urination (de Boer 1977). The familiarity of a given scent mark was negatively correlated with the amount of scenting shown towards it. Fresh marks were explored before older ones. Contrary to de Boer's findings, Natoli (1985) showed that both male and female cats investigated sprayed urine from a foreign male significantly more intensively than excretory urine. This finding was later confirmed by Passanisi and Macdonald (1990). They also found that urine from unknown individuals was investigated more thoroughly than urine from familiar individuals. Explanations for the function of scent marking in the cat have been put forward by Leyhausen (1971). He suggested a system of "traffic lights" where a fresh mark equalled a red signal saying stop - area occupied - do not enter. Similarly an old mark would signal a green light.

Identification of active compounds in scent marks.

There are two extreme schools of thought on how the composition, chemical complexity and mode of action of chemosignals in mammals are to be approached, and the choice will affect the methods used to identifying the biologically active compounds (Albone 1984). One approach is the "response-guided strategy" in which the scent samples are subjected to a series of fractionations by various chemical methods while the biological activity of the resulting fractions are followed by the use of bioassays. This should ideally lead to the isolation of a pure active material which can then be identified by the appropriate chemical methods. The second approach is the "chemical image strategy" in

which the perception of the entire matrix is seen as important for the proper function of scent communication. This school assumes that the perceptual qualities of the entire matrix (a urine mark, glandular secretion, etc.) take on properties above those which can be accounted for by a description of the quantity and quality of the individual components. This school of thought is useful in drawing attention to the possible synergetic effects of the individual components of the scent samples, but it does not suggest any ways of identifying the underlying chemical mechanisms in the mammalian chemocommunicatory systems. Even though the "response-guided strategy" may not be perfect it still presents a productive way in which the chemo-communicatory systems can be investigated as long as one bears in mind that there may be synergetic effects of the compounds which can not be explained by their individual identity and structure.

A number of studies has successfully used the response-guided strategy to identify biologically active fractions or individual compounds for specific mammalian species. These include the house mouse and the bank vole, both of which have been reviewed in the above section on rodents and lagomorphs. For both these species appropriate bioassays were used to test individual compounds or fractions of the scent marks for biological activity. In both species the time spent near the experimental marks and the rate and pattern of scent marking were used as indices in the bioassays. Other indicators of behavioural distinction were the time spent investigating individual marks and the occurrence of flehmen. These indicators were also used by Rasmussen and co-workers in their effort to identify active compounds in the urine and temporal gland secretion of Asian elephants, *Elephas maximus* (Rasmussen *et al.* 1986, 1990, 1993; Perrin and Rasmussen 1994). They succeeded in isolating and identifying the main biologically active compound present in urine of oestrous and pre-ovulating females. The compound, (Z)-7-dodecen-1-yl acetate, is also used by females of more than 126 species of insects to attract conspecific males (Rasmussen *et al.* 1996)!!

The *Panthera* species.

The social life of *Panthera*:

The group living lion (*Panthera leo*):

The lion is the most social of the five *Panthera* species. It is found throughout sub-Saharan Africa and its main habitat is the open savannah. A small population of Asian lions (*P.l. persica*) is found in the Gir Forest Sanctuary in north-western India. The basic social unit of lions is the pride. It comprises the resident females, their offspring and attendant males. The pride is a permanent social unit which exists for many generations. A pride of lions can vary in size from 2-35 individuals, usually with between 2 and 18 adult females, 1 to 7 adult males, and offspring. The pride lives on defended home ranges 20-500 km² in size (Hanby *et al.* 1995). The males may defend a range encompassing ranges of two or more separate female prides. Large prides may be scattered in several groups ("subprides") throughout the pride range (Schaller 1972; Bertram 1978). The home range is defended by roaring, patrolling and scent marking. Lions can distinguish between the roars of familiar and unfamiliar males and females and use this distinction to defend their cubs and their home range by modifying their behaviour according to the context (McComb *et al.* 1993, 1994; Heinsohn *et al.* 1996, Heinsohn 1997). Some females are persistently more active in the defence of the home range than others (Heinsohn & Packer 1995).

The females of a pride tend to come into oestrous together and give birth synchronously with litter size varying from 1 to 6 and a mean of 2-3. The litters are raised communally with the cubs being able to suck any lactating female. Sexual maturity is reached between the ages of 2½ and 3 years for males and 3 and 4 years for females (Smuts *et al.* 1978; Packer & Pusey 1995). When reaching maturity females usually stay in their native pride though some subadult females may emigrate. All maturing males are expelled from their native pride and their future reproductive success depends on whether they are able to gain control over another female pride either singly or in a coalition with other males. Male coalitions can comprise both related and unrelated males (van Orsdol *et al.* 1985; Hanby & Bygott 1987). The maximum reproductive lifespan of females is around 12 years, and male reproductive lifespans are considerably shorter as males are

only reproductively active when in control of a pride. The period of control varies and can last from a few months up to several years depending on the physical strength of the individual or coalition. Usually coalitions retain control of a pride longer than individuals. When a new male(s) takes over a pride the old males are either killed or expelled from the territory and the incoming males are known in many cases to kill the cubs present in the pride at the time of the takeover (Hanby & Bygott 1987).

In a male depleted area in Zambia Yamazaki (1996) found that male were able to mate with females from different prides than their own, and this may have been a consequence of the fact that males had territories which were smaller than the pride territories thus leaving free space for outside males to operate in. This system allows a limited number of males to manage more females than in the traditional system, and it illustrates a certain flexibility in the social organisation of the lion.

The main period of activity is between dusk and dawn when the pride hunts for food (Rudnai 1979). The hunting effort is usually highly co-ordinated between the pride members with each individual having a favoured position and role in the hunt (Stander 1992). Males rarely participate in the hunt but will gain access to the prey killed by the females.

Apart from normal urination and defaecation lions deposit scent marks of the following types: Spray urination, Scrapes with or without urination (in rare occasions also faeces), clawing of branches or trees and rubbing of cheeks against objects. Scent marking is seen in both sexes (Schaller 1972; Bertram 1978).

The solitary *Panthera* species:

The other four *Panthera* species, tiger (*P. tigris*), leopard (*P. pardus*), snow leopard (*P. uncia*) and jaguar (*P. onca*) are all solitary with pronounced territoriality within each sex group. All these species are very evasive and hard to follow in the wild and therefore most of the research which has been carried out on them so far has been based on radio tracking rather than on direct observations.

The tiger (*Panthera tigris*):

The tiger is found in fragmented populations throughout Asia with five living

subspecies being recognised (Bengal tiger, *P.t. tigris*; Siberian tiger, *P.t. altaica*; Indochinese tiger, *P.t. corbetti*; South China tiger, *P.t. amoyensis*; Sumatran tiger, *P.t. sumatrae*). Only the Bengal tiger remains in any significant numbers in the wild. All the other subspecies have been severely reduced by poaching and a loss of habitat. It is estimated that only 150-200 individuals of the Siberian tiger remain in the wild (Jackson 1994). The preferred habitats of the tiger are tall grassland and forested areas (Seidensticker 1976). There is no basic social unit, apart from the individual, as both sexes are solitary and usually aggressive towards same sex intruders. The only regularly reported social unit is that of a female tiger with her immature offspring. Each sex holds and defends a territory from others of the same sex. A male territory is usually larger than, and may overlap more than, one female territory. Female territories may show a small degree of physical overlap but the animals do not associate (Schaller 1967; McDougal 1977). Territory size is probably related to population and prey density, and the Siberian tigers may hold territories as large as 1000 km² for the males (Matjushkin *et al.* 1980). Territories are defended by scent marking, roaring and fighting (Sunquist 1981; Smith 1993).

When the female comes into heat she associates with a male for the duration of the oestrous period (2-7 days). After a gestation period of around 100 days she gives birth to a litter of 1-7 cubs (mean=3). Cubs stay with their mother until 20-30 months old and males usually disperse further than females (Smith 1993). Sexual maturity is reached between the ages of 3 and 6 (Sunquist 1981).

Tigers are mainly active during the night when most of the hunting take place (Schaller 1967; McDougal 1977; Sunquist 1981).

Tigers show the same range of marking types as seen for lions i.e., normal urination and defaecation as well as Spraymarking, Scrapes with or without urine and/or faeces, Clawing and Cheekrubbing. Both sexes scent mark (Schaller 1967; McDougal 1977; Sunquist 1981).

The leopard (*Panthera pardus*):

Leopards are found in both Africa, where they are sympatric with lions, and in Asia, where they are sympatric with tigers. Leopards inhabit a wide range of habitats ranging from semi-desert to alpine zones but they are usually associated with wooded areas where they can protect their kill from other predators by dragging it up into the trees. As seen in

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the tiger intrasexual territoriality is found in the leopard. Male territories are larger than female territories and may overlap more than one of the latter. Typical territory sizes are between 10 and 60 km², but in very dry and marginal habitats they may be as large as 150-250 km² (Myers 1976; Norton and Lawson 1985; Stander *et al.* 1996; Jenny 1996). Territories are defended by scent marking, vocalisation and fighting.

The typical gestation period is around 100 days and females have litters of 1-6 cubs (mean=3). The cubs become independent of their mothers between 15 and 20 months of age at which time they disperse to establish themselves as territory holders. Sexual maturity is reached between 2-3 years (Bailey 1993).

Leopards are mostly active during the night time when most of the hunting takes place (Myers 1976; Seidensticker 1976; Bailey 1993).

Leopards use the same range of mark types as lions and tigers i.e. Urination, Defaecation, Spraymarking, Scrape/urination or defaecation, Clawing and Chinrubbing. Both sexes scent mark (Bailey 1993; Bothma and le Riche 1995, Jenny 1996).

The snow leopard (*Panthera uncia*):

Because of its harsh habitat and shy nature much less is known about the snow leopard than about the other *Panthera* species. The snow leopard inhabits mountainous areas of Asia with an altitude of between 1800 and 5500 m above sea level. Snow leopards are solitary and territorial with individual territories of up to 100 km². There is a breeding season between January and May where males and females may associate and hunt together. Gestation period is around 100 days and litter size is between 1 and 4. The main activity period is at night (Schaller *et al.* 1988; Fox *et al.* 1991; Macdonald 1995).

The snow leopard is known to use Spraymarking and Scrapes in addition to normal urination and defaecation. As there are few trees in its natural habitat it is doubtful whether it uses Clawing. Both sexes exhibit marking behaviour (Schaller *et al.* 1988; Fox *et al.* 1991).

The jaguar (*Panthera onca*):

The jaguar is found in Central and South America and its preferred habitat are areas

with forest and swamps. Jaguars are solitary and maintain a territory of between five and 500 km². In some locations overlap between female territories is seen, and male territories overlap several female territories. Males and females only associate during breeding and the female gives birth to a litter of 2-4 cubs after a gestation period of around 100 days. The cubs disperse at an age of about 2 years and sexual maturity is reached around 3 years of age. As with the other *Panthera* species the jaguar is mainly nocturnal (Schaller and Crawshaw 1980; Rabinowitz and Nottingham 1986; Macdonald 1995).

Jaguars have been found to exhibit the following types of marking behaviour apart from normal urination and defaecation: Scrapes and Clawing. Scrapes are frequently associated with faeces but not with urine. Spraymarking has not been noted by either Schaller and Crawshaw (1980) or by Rabinowitz and Nottingham (1986) but this may be due to the nature of the studies which were both done by radio tracking, and spray marks are not as obvious to human observers as are scrapes or clawings. Both sexes scent mark.

Scent marking in *Panthera*:

Scent marking in the *Panthera* species has been described by a number of researchers. Among the first accounts are Schaller's observations on tigers (Schaller 1967) and lions (Schaller 1972). Both of his books also contain a short entry on leopards. In these accounts the marking behaviour is described and some suggestions put forward as to the possible functions of the scent. These were : 1) scent functions as a trail-marker enabling animals to follow each other's trails, 2) scent is used to mark a range indicating that the area is occupied, and 3) scent communicates identity, time of deposit and reproductive state to passing animals (Schaller 1967). Later studies by Sunquist (1981) and Smith *et al.* (1987, 1989) have elaborated on these findings, especially with regard to territorial marking, and a cost-benefit model was put forward by Smith *et al.* (1989). They suggested that an animal evaluates the risk of encountering another individual by the strength of the local odour field. When the probability of encounter exceeds the benefits gained by using an area, an animal should avoid the area in question.

Controversy has long reigned over the precise origin of the "marking fluid" used by lions and tigers in scent marking. It has been reported by several workers that when these big cats deposit scent marks they include secretions from the anal sacs in the scent mark (Lion: Schaller 1972; Bertram 1978, Tiger: Schaller 1967; McDougal 1977;

Brahmachary and Dutta 1981). This conception was formed on the basis of the observation of "a granular, whitish precipitate ... apparently a secretion from the anal glands" being present in the marking fluid of the tiger by Schaller (1967). This misconception was finally rectified by Brahmachary and Dutta (1987) when they concluded that as there is no connection between the anal glands and the urinary tract (Hashimoto *et al.* 1963), the anal gland secretion can only mark the faeces and not the urine. The "whitish precipitate" must therefore originate somewhere from inside the urinary system. In 1947 Hewer *et al.* investigated the contents of the bladder of a dead tiger and they found substantial amounts of lipids in the bladder urine. They also found lipids in the urine collected from the floor of night cages housing tigers of both sexes. They estimated that tigers excrete as much as 20 grams of lipid matter a day, and that the concentration of lipids in the urine is correlated with the kidney fat index rather than with the level of lipids in the blood (Hewer *et al.* 1948). Asa (1993) carried out a series of analysis on bladder urine extracted from anaesthetised animals which confirmed that the bladder urine of tigers contain lipids in the form of a whitish substance. In addition she found lipids in the bladder urine of lions, but she did not find evidence of lipids in the bladder urine of either leopard, puma (*Felis concolor*) or cheetah (*Acinonyx jubatus*).

As lipids are present in the bladder urine they will be released both by Spraymarking as well as by normal Urination. It is therefore unlikely that their presence in scent marks is the result of a voluntary process involved in the motor pattern of scent marking. It is also doubtful whether an animal is able to control the ratio of "lipid to urine" deposited in a scent mark. Therefore no circumstantial or conclusive evidence has been presented so far for the addition of any secretions to the bladder urine before it is deposited as a scent mark. Against this background it is reasonable to assume that the bladder urine from an animal and therefore its urine produced during a normal urination, will contain exactly the same range of chemical compounds as that found in the scent mark once it is deposited. It follows that the urine collected from a normal urination should contain the same information as a Spraymarking mark, and should therefore be just as valuable to use in a chemical analysis of the compounds involved in the scent communication of these species.

The urine (marking fluid) of the tiger has been the subject of a few chemical investigations. Brahmachary & Dutta (1979, 1981, 1987) performed a chemical analysis which showed that the major component of the tiger urine is phenylethylamine. This finding, however, has been questioned by Banks *et al.* (1992) who found only minor traces of phenylethylamine in samples of urine from two tigers. They found a range of other amines with triethylamine being the most pronounced.

These are only first steps in the process of identifying the active components and decoding the message of the marking fluid, which at present can only be guessed at. The composition and function of the marking fluids of the lion and leopard have been even less studied than that of the tiger. Other aspects which have to be taken into account when investigating scent communication of these species are the differences in social organisation and habitat preference between the species outlined in the previous section.

On the basis of our knowledge on group structure and territoriality of the large *Panthera* species as well as the information from studies of scent communication in other and related species a number of hypotheses concerning scent communication of lions, tigers and leopards can be formulated.

Hypothesis 1: The scent marking rates of animals holding a large territory must be higher than an animal holding a small territory if the density of marks are to be equal. The prediction following this hypothesis is that males will generally have higher rates of marking than females because of their larger territories.

Hypothesis 2: A second hypothesis is that individuals living in a group-defended territory should have a lower marking rate than those holding their own exclusive territory, if the sizes of the territories are comparable. Hypothesis 2 predicts that female tigers and leopards will have higher marking rates than female lions because in these two species the females defend a territory alone whereas lions defend a group territory.

Hypothesis 3: As has been mentioned earlier in this Introduction the marking rates of castrates has been found to be lower than those of intact males for a number of species, it is therefore reasonable to assume that the same will be true in the *Panthera* species. Therefore one would predict that castrated *Panthera* males will have lower marking rates than those of intact males.

On the chemical level one can also put forward a number of hypotheses.

Hypothesis 4: In order for scent marking to play any role at all in communication it is hypothesised that an individual of any of the three species will be able to distinguish between the scents of two other individuals of the same species. The prediction from this hypothesis is that there will be a (measurable) difference in the

These are only first steps in the process of identifying the active components and decoding the message of the marking fluid, which at present can only be guessed at. The composition and function of the marking fluids of the lion and leopard have been even less studied than that of the tiger. Other aspects which have to be taken into account when investigating scent communication of these species are the differences in social organisation and habitat preference between the species outlined in the previous section.

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reaction of an animal to scent marks from different individuals.

Hypothesis 5: It can further be hypothesised that the ability to distinguish between two marks is based on differences in the chemical composition of the scent marks. It therefore follows that a chemical analysis should reveal differences in the composition of individual scent marks either at a qualitative (differences in compound composition) or a quantitative (differences in concentration of identical compounds) level or a combination of both.

Hypothesis 6: Another biologically meaningful distinction which the animals should be able to make is the difference between scent marks from males and females. This difference would be based on the same criteria as Hypothesis 5.

Hypothesis 7: Males would also be at an advantage if they could distinguish between the scent marks left by a female in oestrus and a non-oestrous female. Again this predicts a measurable difference in the chemical makeup of the scent marks.

Hypothesis 8: It would also be an advantage for an animal to be able to distinguish between the marks from another individual of its own species and that of a related species. Chemical foundation for this ability should be present in the scent marks.

No studies have yet attempted to investigate the dynamics of scent marking behaviour in the large *Panthera* species, but the project presented here is an attempt to address the relevant central questions by investigating the hypothesis listed above.



CHAPTER 2: MATERIAL AND METHODS.

This chapter considers the study animals used, describes the enclosures available to them, provides a definition of the behaviours studied and describes the procedures for the collection of data and scent mark samples. Furthermore the procedures for the experimental work are explained, and the equipment and methods used for chemical analysis are described. All figures referred to in the text can be found at the end of the chapter on pages 44 to 48.

The study was conducted on groups of captive animals, and the testing of the hypotheses was structured in the following way. Firstly the activity and social environment of the study groups was assessed in order to detect any influence these factors may have on the marking behaviour of the animals. Secondly the undisturbed marking behaviour of the animals was observed in order to provide background information against which the results of the experiments and chemical analyses could be interpreted. Thirdly a series of experiments was conducted in which the animals were presented with scent samples from different individuals varying in sex, age, reproductive status and species of scent donor. These experiments were conducted both in a "natural" setting in the enclosures as well as in a more artificial set-up in the night cages. Finally the scent samples collected were subjected to chemical analyses. The results of the analyses were used to construct lists of the compounds present, as well as their concentration, in the different individuals and species. These lists were used to detect any differences in composition of the samples between individuals or species, or any variations in concentration of the constituents in the samples.

Location of study sites.

The study animals were located in three different Danish Zoos or Safariparks (**Figure 2.1**). One was Copenhagen Zoo, which is an urban zoo located in the capital of Denmark, Copenhagen (København). The second was Givskud Safaripark, which is situated in Jylland. The third study site was Knuthenborg Safaripark, located on the island of Lolland, between Sjælland and the north German coastline. Three additional Zoos provided urine samples from individuals of the study species. These were Aalborg Zoo in

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Jylland, Odense Zoo on Fyn and Kolmaarten Safaripark in Sweden.

Study Animals.

The animals used in this study are listed in the **Tables 2.1-2.3**, with details of location, the name by which they will be referred to in this thesis, the sex of each animal, its date of birth, and finally comments on the parents' identity (if known) and events in its life relevant to this study. At the time of planning this project one more lion pride was available in Givskud. However in order to avoid inbreeding the Zoo decided to take out the old male of the pride and introduce a new young male. Unfortunately this introduction failed and the pride was left without any males, so the observations on this pride was discontinued. Furthermore the male tiger Amur in Knuthenborg, which was still intact during the planning of the project, was castrated because of social unrest which had suddenly erupted in the group. This meant that the total number of study animals which was available during the planning phase of the project was reduced by one lion group and one intact male tiger during the early phase of the fieldwork period. The period of fieldwork extended from June 1993 until August 1995.

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Copenhagen Zoo (KBH).

Table 2.1a : Lions *Panthera leo*

Name of animal	Sex	Date of birth	Age at start of study	Comments
Willy	M	10/10-87	6 years	From Boraas Zoo (Sweden)
Nanna	F	03/01-89	4 years	Born in KBH
Elsa	F	03/01-89	4 years	Born in KBH
C1.1	F	08/06-94	0 years	Litter 1, Parents are Willy and Elsa
C1.2	F	08/06-94	0 years	Litter 1, Parents are Willy and Elsa
C2.1	F	08/10-94	0 years	Litter 2, Parents are Willy and Nanna
C2.2	F	08/10-94	0 years	Litter 2, Parents are Willy and Nanna

Table 2.1b : Siberian tigers *Panthera tigris altaica*

Name of animal	Sex	Date of birth	Age at start of study	Comments
Sym	M	08/10-91	2 years	From Marwell Zoo (UK)
Mercedes	F	27/03-91	2 years	From Koln Zoo (Germany)

Table 2.1c : Leopards *Panthera pardus*

Name of animal	Sex	Date of birth	Age at start of study	Comments
Sorte	M	09/04-91	2 years	From Wuppertal Zoo (Germany)
Plet	F	04/07-90	3 years	Born in KBH
Blacky	M	01-95	0 years	Parents are Sorte and Plet
Spot	M	01-95	0 years	Parents are Sorte and Plet

Givskud Safaripark (GIV).

Table 2.2 : Lions *Panthera leo*

Name of animal	Sex	Date of birth	Age at start of study	Comments
Pride 1:				
Hubert	M	21/06-83	10 years	From Limburg Zoo (Germany), Died 06/01-94
Flop	M	27/06-90	3 years	From Rotterdam Zoo (Holland), Introduced to pride 02-94, Brother of Nero.
Nero	M	27/06-90	3 years	From Rotterdam Zoo (Holland), Introduced to pride 02-94, Brother of Flop.
Linda	F	23/02-85	8 years	Born in GIV
Fifi	F	21/05-85	8 years	Born in GIV
Flora	F	21/05-85	8 years	Born in GIV
Fie	F	09/05-90	3 years	Born in GIV, Fathered by Hubert
Fiona	F	09/05-90	3 years	Born in GIV, Fathered by Hubert
Lene	F	18/06-90	3 years	Born in GIV, Fathered by Hubert
Frida	F	16/11-80	13 years	From Boraas Zoo (Sweden)
Lillian	F	21/12-92	1 year	Born in GIV, Fathered by Hubert
				+ 10-12 cubs of 0-2 years of age which are included in the data as a group.
Pride 2:*				
Polle	M	24/01-84	9 years	From Boraas Zoo (Sweden), Removed from pride 01-94
				* This pride also included 6 females, but as the male was removed from the pride shortly after the start of this study and the introduction of a replacement male was unsuccessful, the observations on this pride was discontinued and the effort concentrated on Pride 1.

Knuthenborg Safaripark (KNU).

Table 2.3 : Siberian tigers *Panthera tigris altaica*

Name of animal	Sex	Date of birth	Age at start of study	Comments
Amur	M	27/03-88	5 years	From Howletts Zoo (UK), Castrated 12/04-94
Jahrin	M	25/03-80	13 years	Castrated as cub
Amba	M	27/03-88	5 years	From Howletts Zoo (UK), Castrated as cub
Majenka	F	05/09-80	13 years	
Pepezja	F	25/03-80	13 years	Sister to Jahrin
Unda	F	05/07-84	9 years	
Kacug	M	10/11-92	1 year	Parents Amur and Majenka, Castrated 1993, Brother to Abakajn
Abakajn	M	10/11-92	1 year	Parents Amur and Majenka, Castrated 1993, Brother to Kacug
Sibir	M	02/04-93	2 months	Parents Amur and Unda, Castrated 12/04-94, Brother to Ruslan
Ruslan	M	02/04-93	2 months	Parents Amur and Unda Castrated 12/04-94, Brother to Sibir

Hours of observation of activity and behaviours:

The number of hours of observation was 1190 spread between species and Zoos as follows:

Copenhagen Zoo: Lions: 250 hours
 Tigers: 190 hours
 Leopards: 120 hours

Givskud Zoo: Lions: Pride 1 (Hubert) 25 hours
 Pride 1 (Flop+Nero) 300 hours
 Pride 2 (Polle) 25 hours

Knuthenborg Safaripark: Tigers: 280 hours

Study sites.

Copenhagen Zoo (KBH).

Lion enclosure: (Figure 2.2 on page 45)

The outside enclosure is T-shaped, with the upright of the T being surrounded by a moat. On the other sides concrete walls have been erected, with two hatches at the back leading into the night cages. The surface area of the outdoor enclosure is approximately 450 m² and is covered with earth and gravel. Additional features are provided by a number of boulders, an arrangement of tree trunks and two live trees, one of them with a wooden platform at a height of about 2 m. The night area comprises five separate but connected night cages. There is a keepers' corridor running along the back of the cages.

Tiger enclosure: (Figure 2.3 on page 46)

The tiger enclosure is about 280 m² in size. To the south and west, a moat and a low concrete wall borders the viewing area. The north and east sides are protected by tall concrete walls. The north-eastern part of the enclosure is level, whereas the southern and western parts slope down towards the moat. There is a small stream running through the enclosure. West of the tree trunk a concrete platform extends towards the moat, with a grotto underneath. A tree trunk, some boulders and bushes provide further structuring. In the north-eastern corner a double fence and dense bamboo vegetation divides the tiger and leopard enclosures. In the northern wall there are three hatches giving access to the night cages which comprise two small and one large cage, but the big cage is mainly used when a female has cubs. There is a keepers' corridor running along the back of the cages. The tigers are housed in visual, auditory and olfactory contact with the leopards and snow leopards.

Leopard enclosure: (Figure 2.3 on page 46)

The surface area of the leopard enclosure is roughly 100 m². There is fence on all sides as well as an overhead wire mesh roof at a height of approximately 5 m. The enclosure is

hexagonal in shape. The south-west corner borders the tiger enclosure, and the north-west corner the snow leopard enclosure. The surface is covered with gravel and earth with some sparse grass in places. In the northern end there is a small stream running from the leopard enclosure through the snow leopard enclosure. Several upright and fallen tree trunks provide structures for three-dimensional movement. Boulders are also present. Ropes have been suspended from some of the tree trunks to provide play opportunities. In the western wall a single hatch gives access to the two night cages. The leopards are housed in cages adjacent to those of the tigers and snow leopards.

In KBH Zoo the outside areas of the animal enclosures are cleaned every morning by the keepers while the animals are locked in the night cages. They are readmitted to the outside area when the cleaning/maintenance is over. Every night the animals are given access to the cages by the opening of a hatch.

The cats are fed a diet of meat every day except Mondays. The main type of meat is beef, but it is regularly substituted by pork, poultry or game. The feeding takes place in the outside enclosure at 1 p.m. for the lions and at 2 p.m. for both tigers and leopards. Each animal gets on average 2 - 6 kg of meat per day, depending on the species of cat.

Givskud Safaripark (GIV).

Lion enclosure: (Figure 2.4 on page 47)

The enclosure of pride 1 is the largest enclosure in Givskud. This drive-through enclosure covers an area of about 4 ha (40.000 m²) and is roughly quadrangular in shape. A gravel road runs through the enclosure in the shape of an U with the southern arm dividing into two for part of the way. The entrance and the exit is via the Pride 2 enclosure which borders the Pride 1 enclosure at the eastern end. The middle section of this border is blocked visually for the lions by plywood boards mounted on a double fence. The road entries are constructed of 5 m wide cattle-grids with electrical wires between the bars for the first metre on each side. The enclosure is fenced by a 2½ m tall double structure with barbed wire and electrical wire running on the inside. A large part of the enclosure is covered with vegetation in the shape of pine and fir trees together with shrubs. There are also a few deciduous trees. Uncovered areas of grass and sand are found along the north border, in the north-western corner, and along the south border. There are three water-holes in the north-western end. In the western end are two slopes running

west-east, but they gradually level out towards the east. From the south-western corner the lions can overlook one of the savanna enclosures where wildebeest (*Connochaetes taurinus*), sable (*Hippotragus niger*), oryx (*Oryx gazella*) and zebra (*Equus burchelli*) graze. The night house is located in the north-western corner. It comprises 10 individual cages, which are all linked by small doors. The lions are locked in these cages as a group during the night, although lionesses with young cubs usually get a cage to themselves. A keepers' corridor runs along the back of the cages.

During the Summer season (April - October) the lions are let out into the enclosure just before 10 a.m., and are locked in the night cages around 8 p.m.. In the Winter season (November - March) they are let out at about the same time, but they are locked in around 3 p.m.. This is because the park is closed to the public at that time of year. The lions are fed as much as they can eat once a week in the night cages after being let in. This is usually done on Wednesdays. On feeding nights the males are locked in separate cages, while the females are kept together as a group. The main part of the diet is beef.

Knuthenborg Safaripark (KNU).

Tiger enclosure: (Figure 2.5 on page 48)

This drive-through enclosure is about 3 ha (30.000 m²) in area, and is roughly rectangular in shape. There is a 2½ m tall double fence running around the periphery, of barbed wire and electrical wires. A B-shaped gravel road runs through the enclosure, with a single entrance in the south-western corner with electrified double gates. Parts of the enclosure are largely uncovered with only grass vegetation. The other parts are covered with trees and shrubs of pine, fir, oak and birch. There is a water-hole in the northern part of the enclosure. Tree trunks for climbing and playing are present. At the southern end are the night cages and four outdoor cages bordering the main enclosure. There are eleven night cages, all connected by small hatches. A keepers' corridor runs the length of the back of the cages. The tigers are kept in separate cages during the night, except for young cubs, which are allowed to be together. The tigers are fed every other day on a diet of beef, pork, poultry or game, with each tiger being given approximately 5 kgs.

All the males are allowed out together in the main enclosure every day, but only one of the three females is let out at any one time. The other two are kept in the outdoor cages bordering the main enclosure. This procedure is to prevent any hostility between the females.

The tigers are let out just before 10 a.m. every day during the opening season (April - October), and readmitted to the night cages at 5 p.m.. Out of season they are allowed out for 4 or 5 hours a day.

Observation methods.

The behavioural observations comprised three different aspects namely the activity of the animals, their social interactions and their marking behaviour, with the last part being subdivided into scent mark deposition and response to scent marks. Data on activity and social interactions were collected to assess the basic behaviours of the focal groups compared to those of wild animals, and thus to evaluate any influences the captive environments might have on the animals. The two categories of marking behaviour were investigated in order to collect data which could test Hypotheses 1 - 3.

In KBH the observations were made from the public area outside the enclosures. In GIV and KNU they were made from a vehicle within the enclosure, which made it possible to follow the animals as they moved around. A pair of 10 X 24 binoculars were used during observations when necessary. In the small enclosures in KBH all the animals of a group could be observed at the same time. In GIV and KNU this was possible for most of the time, but when the group split up, part of the group was chosen and followed until all the animals were within sight again. In GIV and KNU observation periods occurred during the opening season (late April to beginning of October) when the animals were out for the whole day (10 a.m. till 5 p.m.). For the sake of comparison the outdoor observations in KBH were also concentrated during the summer season, and the winter season was spent in indoor experimental work and chemical analysis.

Data on the activities of the animals and the social reactions between individuals were collected to provide information on the social relations within the focal groups. A scan sampling of activity for all individuals was made every 10 minutes, and social reactions observed were scored within these intervals. In order for the data collection to be usable, it was necessary to identify all group members individually.

All adult individuals of all the study populations were individually recognisable. For lions recognition was based on the following criteria : sex, colour variations, scars, pattern of whisker spots and pattern of pigmentation on the nose. Cubs less than 2 years old could not be individually identified and were pooled in one group. For tigers and leopards identification was made by noting differences in coat pattern and colour.

Activity.

Activity data were compiled by noting the activity for each individual every 10 minutes. The categories of activity are defined as follows :

Moving : The animal is moving around the enclosure. Includes both walking and running.

Standing : The animal is supporting itself on all four legs, but without moving around.

Sitting : The animal is resting on its hind quarters with its forelegs extended vertically.

Lying : The animal is reclining either on its sternum, its side or on its back.

Other : The animal is engaged in an activity not included in the above categories. (e.g. : social interactions, drinking, hunting birds or squirrels, or scent marking.)

Social behaviour.

Social interactions between individuals were noted continuously, with the type of behaviour, the actor and the receiver being recorded. The categories of social behaviour used are defined as follows :

Headrubbing : One animal approaches another and rubs either its cheeks or forehead on the other animal's cheeks or forehead.

Bodyrubbing : An animal slides along the body or head of another animal rubbing its body against it in the process.

Allogrooming : One animal licks any part of another animal.

Playing : Two or more individuals interact in a playful manner, i.e. without threatening behaviour involved and without direct aggression. Play may take the form of chasing, jumping at, wrestling and playing with objects such as sticks, stones, bones or vegetation.

Investigating : An animal sniffs an area (usually the genital area) of another animal.

Aggression, Mild : All types of threats, intention movements of striking with paws or biting with jaws, but without actual contact being made with the receiving animal.

Aggression, Serious : Actual contact with either paws or teeth with the receiving animal.

Mating : Copulation, as well as invitations to this. For males sniffing the female genital region followed by mounting attempts. For females sliding along the body of the

male from back to front, and then crouching in front of the male presenting slightly raised hindquarters.

Information on scent marking was collected in three different ways. Firstly by observing the undisturbed marking behaviour of the animals in their outdoor enclosures. Secondly by carrying out a series of experiments, both indoor and outdoor, in which the animals were presented with a range of scent marks from unknown individuals of either the same or a different *Panthera* species. Thirdly by chemically analysing the scent marks collected to determine the composition of compounds and their relative concentrations.

Marking behaviour.

Marking behaviour was recorded as it occurred. Both active scent marking and responses to other scent marks were noted. Information collected included identity of marking / responding individual, time and place of action, type of mark deposited or type of reaction to mark and details on any other information which seemed relevant in the given context. The following marking categories were used :

Spraymarking : A spray of urine/markings fluid is ejected from the urinary tract of the marking animal while it stands with the hindquarters towards the marked object with tail lifted vertically. The marked object can be a tree, a stone, a wall, a fence, tufts of tall grass or other vertical objects.

Clawing : The marking animal drags its claws along the marked object. The only objects marked in this fashion are trees or branches.

Scrape/urination : The animal makes a shuffling movement with its hindpaws while standing in the same location. During or after this it deposits up to a few jets of urine. This type of marking is done on earth or in grassy areas. (A rare variation of this marking type is Scrape-defaecation, where faeces are deposited on top of the scrape instead of urine)

Chinrubbing : The animal rubs its cheeks along the surface of the object repeatedly. It may alternate between the cheeks or it may do it with only one cheek. Surfaces marked in this way are tree trunks, branches, walls and fence posts.

Defaecation : An animal defaecates on earth, grass or other surface.

Urination : An animal squats on the ground and releases a stream of urine from its urinary

tract.

The following response categories were used :

Scenting : The animal sticks its nose close to the mark investigated and keeps it there for several seconds.

Flehmen : This familiar grimace is thought to play a role in the testing of chemical substances by way of the Jacobson's organ. It may be performed several times in succession.

Licking : The animal licks the place where the scent mark is located.

Overmark : The animal, after having scented a mark deposits its own mark on or close to the investigated mark.

Experimental work.

The experiments described in the following section were carried out in order to gather information which would help in the investigation of Hypotheses 4 - 8. The scent presentation experiments provide information on whether an animal's response to a given scent mark or class of scent marks can be used in the identification of the scent mark with regards to identity, age/sex group, reproductive status or species. The indoor experimental procedure with four marks, three from one individual and one from another, being presented at the same time, was designed particularly to test Hypothesis 4.

Sample collection.

Collection of scent marks had to be adjusted to the individual conditions in the enclosures and cages. For the KBH populations samples were collected mainly from the floor of the night cages, but for the male tiger, additional samples were obtained from the outside enclosure by placing small test tubes in specially drilled holes in the wall at one of his marking sites. In GIV samples were also collected from the floor of the night cages, but as these were mostly covered in sawdust, a method had to be devised for extracting the samples from the sawdust. This was done by constructing a oversized garlic press of stainless steel which could hold about one litre of sawdust, and by compressing this, the fluid can be extracted and collected in test tubes. In KNU the samples were again collected

from the floor of the night cages. Between the time of deposit by the animal and collection by the author all samples were subjected to natural rates of evaporation. This condition was similar for all samples and so any effect the evaporation process may have had on the samples would be identical for them all. Hence evaporation should not influence a comparison between the samples.

All samples were stored in identical plastic test tubes with a holding capacity of 10 ml. The samples were frozen immediately after collection and kept at -18°C until the time of experimentation and analysis.

Additional samples were provided by Odense Zoo, Denmark, from a 5-year-old Siberian male tiger (Ramos), by Aalborg Zoo, Denmark, from a 7-year-old male lion (Napoleon), a 7-year-old male tiger (Rubi), two female tigers, one of 9 (Vela) and one of 3 years of age (Felina), a 2-year-old male leopard (Tabiz) and a 3-year-old female leopard (Urmia), and by Kolmaarten Safaripark in Sweden from an adult male lion (KolmHan). These samples were also obtained from the floor of the night cages, and stored in test tubes provided by the author.

Experimental design.

The species, sex and age characteristics of the scent sample used in any given experiment depended on the availability (i.e. which scent samples were in stock) of scent samples on that particular day. In all the experiments distilled water was used as a neutral control.

Outdoors :

On each experimental day, I entered the enclosure together with the keeper when he carried out his cleaning in the early morning. The thawed scent sample was sucked from the test tube into a disposable syringe, and then deposited on the chosen site. Sites used for mark presentation were vegetation (bushes or low hanging branches), rocks, tree trunks, and the peripheral walls. Depending on enclosure size and number of animals, one or more marks were deposited. After I had left the enclosure, the animals were readmitted, and direct observations were carried out for four hours after the discovery by the animal of the mark.

Indoors :

Before the animals were readmitted to the night cages in the late afternoon, experimental marks were deposited inside the night cages, and a time lapse video recorder

was placed outside the cage in the keepers' corridor to record the reactions of the animals for the duration of the night. The recording was analysed the following day and the animals' reactions to the experimental marks noted.

The marks were deposited using the same procedure as for the outside experiments. The site of deposit of the experimental marks varied from cage to cage depending on their construction. In the KBH lion cage, the samples were deposited on the wall covering of white ceramic tiles at a height of about 1 m. Four marks were placed next to each other with a mark to mark distance of 50 cm. The same procedure was used in the leopard night cages. In the tiger night cage in KBH, the marks were deposited along the rim of a wooden resting platform mounted on one of the walls about 1 m above the floor. The same mark presentation pattern as in the lion cage was used. In the GIV cages, the marks were deposited on the horizontal part of a steelbar running along the front of the cages. The elevation of the steelbar was approximately 1 m. Again four marks were placed with a distance of about 50 cm between each mark.

The sites where the marks were deposited were cleaned every morning so as to neutralise any scent left from the previous night's mark. No indoor experiments were carried out in KNU because of social unrest in the tiger group.

Chemical Analysis.

Various methods have been used by different workers in an attempt to identify the active components in the urine used by the big cats when scent marking. Thin Layer Chromatography (TLC), Paper Chromatography and Gas Liquid Chromatography (GLC) has been used by Brahmachary and Dutta (1979, 1981, 1984) in their analysis of the urine of the tiger and leopard, whereas Banks *et al.* (1992) chose to use Gas Chromatography (GC) fitted with a nitrogen-specific detector in their analysis of amines of tiger marking fluid. The choice of pre-analysis treatment of samples and the analysis method will influence the results obtained. If GC is chosen, the choice of column, temperature program and detector type will also influence the outcome. To get a complete picture of all the compounds contained in a scent sample one would have to perform a number of analysis, each specifically directed towards a sub-group of compounds. One has to remember that the filters we use in analyses to detect and identify chemical compounds in the scent samples may work in different ways from those used by the animals. So even

though we might succeed in identifying and quantifying all compounds contained in a scent sample, it will still be very difficult to pinpoint which of these compounds are important for the animal investigating the mark. The relative concentration of compounds does not necessarily reflect their relative importance to the animal, and indeed a combination of compounds may cause a reaction which could not be obtained by any one of them alone. These limitations should be kept in mind when performing a chemical analysis of biological matter and interpreting the results.

The chemical analyses presented below were carried out in order to look for the chemical basis for discriminating between two different scent marks, and thus provide data which would help in the investigation of Hypotheses 4 - 8. The data collected in this part of the study could be used in explaining the chemical mechanisms underlying any behavioural differences found in the experimental scent presentations in the response to different classes of scent marks.

The number of scent samples analysed for the different species and sexes were as follows:

Lions:	18 from five adult males
	1 from a male cub
	43 from thirteen adult females
	1 from the bladder of a dead subadult female via catheter
Tigers:	7 from three adult males
	4 from two subadult castrates
	8 from four adult females
Leopards:	10 from two males and two females

The analyses were carried out by the author using facilities provided by the Danish Technological Institute, DTI Chemistry Technology in Taastrup, Denmark.

All chemical analyses were performed on a combined Gas Chromatograph - Mass Spectrometer (Fisons MD 800). 1 ml of undiluted scent sample was introduced into a sample glass with appropriate labelling, which was then placed in the holding tray of the Headspace autosampler of the chromatograph. The analysis was made using the "Headspace" analysis method, in which a sample of air from the sample glass (the "Headspace") is injected into the injection port of the chromatograph. The carrier gas used was Helium at an inlet pressure of 5 psi.

The technical specifications of the GC Capillary Column were: Column I.D: J & W, DB624 (4320732), Film thickness : 1.80 μ m, Column dimensions : 30 m X 0.315 mm,

Temperature limits : -20°C to 260°C, Coating : 6 % Cyanopropylphenyl and 94 % Dimethylsilicone.

The analyses were run automatically using the MassLab (v1.1) computer program. The Chromatograph was run in two different modes. The first was a split-mode with a split ratio of 1:5 and the following additional parameters: Injector temp 200°C, Initial oven temp. 40°C for 1 min, then increased by 10°C/min up to 200°C and this temperature was maintained for 8 min. The second was a splitless-mode with the following parameters: Injector temp 200°C, Initial oven temp. 40°C for 1 min, then increased by 20°C/min up to 200°C where it was maintained for 16 min. In both modes the scent sample glass was preheated for 1 hour at 70°C before the headspace sample was taken and injected into the chromatograph, in this way the compounds in the sample evaporate and form a state of equilibrium with the air above the sample. The amount of sample air injected in each case was 1 ml.

The Spectrometer was run with the following parameters: Type : FULLSCAN, Ionisation mode EI+, Mass range: Start: 20.00 End: 350.00.

When transferring the scent sample into the sample glass it is inevitable that molecules present in the laboratory air at that particular time also become included in the sample. These laboratory air compounds will then contaminate the test sample by appearing as peaks on the chromatogram resulting from the analysis. In order to minimise the impact of this contamination on the final results, a set of two laboratory air samples were also analysed each time a test series was analysed. The peaks caused by the compounds in the laboratory air can thus be subtracted from the chromatograms of the test samples. Another possible source of contamination for most of the lion samples are compounds originating from the sawdust from which the urine samples were extracted. To eliminate the influence of this contamination on the results, three extractions of distilled water on sawdust were made, one being allowed to soak in the sawdust for one hour, the second for three hours and the last for five hours. These extractions were then analysed using the same procedure as for the urine samples, and the resulting chromatograms made it possible to identify compounds with a probable origin from the sawdust and thus exclude those compounds from the further data analysis.

The results from the analyses were automatically stored on disks in individual files. These were later analysed using the MassLab programme package, and a chromatogram for each sample was produced. From each chromatogram the mass spectrum for all peaks was extracted. These mass spectra were used in the identification of the individual compounds in the samples. The identification was made on the basis of the procedures

described in McLafferty and Tureček (1993) and with the help of the NIST/EPA/NIH Mass Spectral Database v4.0, available at DTI.

The Total Ion Count (TIC) was extracted for each of the peaks on the chromatograms. The TIC is a quantitative measure of the amount present for each of the peaks and its value is obtained by approximating the area under the peak. The TIC values were used to approximate the percentage content of each compound in a sample. Both the areas (TIC) and the percentages were used in a quantitative analysis of differences between the sexes.

When comparing the scent samples the concept of "Overlap" is used in some of the analyses. An Overlap is defined as the percentage of the total number of compounds present in an individual sample which are common to both individuals (or samples) being compared.

Statistical procedures.

In this thesis both parametric and non-parametric tests were used to analyse relevant parts of the data. Because of the low sample size in some of the material presented here special caution has to be applied when interpreting the results of the tests.

In the sections on activity and social behaviour a multi-way χ^2 -test has been used in the comparison of sex/age groups. This type of test has the advantage over a general linear model (GLM) test that it is possible to weigh the input and thus take into account any differences in the number of observations in the different categories. The calculations were done using the SAS statistical package (SAS Institute Inc. 1989a).

When percentages were compared the G-test was used instead of the χ^2 -test because it is better suited to percentage data (Sokal & Rohlf 1995).

In several instances the means of two groups or populations were compared and in these cases a t-test has been used. Again the calculations were done on the SAS software. This program calculates whether the variances of the two groups being compared are significantly different in a F-test, and it provides statistics for situations of both equal and unequal variance. If variances are equal then the normal t-test procedure is followed, but if they are unequal then the approximate t-test is used and the Satterthwaite Approximation is used to calculate the degrees of freedom (SAS Institute Inc. 1989b).

The results of the scent presentation experiments have been produced by a special statistical method developed by Jon Stene of the Institute of Statistics at the University of

Copenhagen, and it is described in Appendix 2. The method is based on the assumption that the number of reactions can be considered as Poisson distributed with a frequency parameter depending on the experimental conditions. The frequency parameters for different experiments are compared two and two based on exact, statistical methods for comparing frequency parameters of two Poisson distributions in contrast to approximative methods based on the χ^2 -distribution. Exact methods are necessary because of the small number of observations in many experiments.

Cluster analysis was used when looking for hierarchical structures and groupings based on the chemical composition of urine samples. The Average Linkage method was chosen as the most robust cluster procedure (SAS/STAT 1990a; Gnanadesikan & Kettenring 1989).

The problem of small sample size.

In investigations of the behaviour of a particular species the most accurate result would be obtained by collecting the relevant measures from every single individual within that species. This is seldom feasible, however, unless the population in question is very small. It is, therefore, usually necessary to limit the number of individuals involved in the research, and the lower the number, the higher the risk of basing conclusions on results that reflect individual variation rather than the true variation within the population as a whole. The individual differences have two effects on the statistical aspects. First, the power of the statistical tests used is reduced because of the increased amount of variability within each group. This makes it less likely that true effects will be detected. Secondly, individual differences make it more difficult to draw firm conclusions about groups from the characteristics of individuals and *vice versa*.

The number of individuals available for a given study varies but generally speaking the larger the body size the fewer there are available. Researchers working on small rodents have an almost unlimited access to individuals and usually have no problems over handling and manipulating the animals. Researchers working on large or rare mammals are often faced with a great limitation in the number of individuals available, and because of their size and other physical attributes, these animals can be dangerous to handle directly. This makes the interpretation of the results obtained a much more difficult task, but with care and careful planning this type of study can still produce worthwhile results.

There are five ways in which the problems caused by low sample size can be alleviated (Martin & Bateson 1993, Martin & Kraemer 1987). The first and most obvious one is to increase the sample size but unfortunately this is not always practically possible because of constraints in access to the animals or in the time limits of the study. The four alternative methods are: (a) to obtain repeated measurements of each individual's behaviour after the experimental (or control) treatment has been applied (repeated outcome scores); (b) to measure each individual's behaviour before, as well as after, the treatment is applied (baseline score); (c) to use a combination of (a) and (b) (repeated baseline and outcome scores); or (d) to measure each individual's behaviour repeatedly across time, over the duration of the response (longitudinal design). In the present study methods (a) and (c) have been applied wherever possible.

There are special cases in which a sample size (N) of 1 is generally accepted as being sufficient (Still 1982) e.g. when the question being asked is whether a particular species possesses a certain discriminatory capacity or whether a particular behaviour is part of a species' repertoire. Examples of these types are Kruuk *et al.* (1984), who trained a single badger to discriminate between the subcaudal gland secretion of other individual badgers, and Gaughwin (1979), who established the occurrence of flehmen in the hairy-nosed wombat (*Lasiorninus latifrons*) from the behaviour of a single individual. But even when a more general description of both qualitative and quantitative behaviours of larger mammals is involved, it is not unusual to find sample sizes of 2 to 6. Thus Beier & Wartzok (1979) deduced the mating behaviour of the species from observations on a single pair of spotted seals (*Phoca largha*). Mills *et al.* (1980) obtained the majority of their data on the scent marking behaviour of the brown hyaena by observing a single group of animals (three males and two females) on their group territory. Gosling (1981) based his discussion of the economics involved in the demarcation in a gerenuk (*Litocranius walleri*) territory on observations on just one adult, territory-holding male. Kruuk *et al.* (1984) supported their accounts of the scent marking behaviour of the badger with observations made on a single captive group comprising one adult male, one adult female and up to four of their offspring. In 1987 Mills and Gorman reported on the territorial marking behaviour of the spotted hyaena, basing their results on the observations made in one territory containing a single group of hyaenas with "one or two adult males, usually four, but up to six adult females and between three and seven subadults". The territorial scent marking behaviour of the European mole (*Talpa europaea*) was described on the foundation of data collected on a lone male by Gorman and Stone (1990), who also

conducted scent presentation experiments on three male and two female moles.

It is thus evident that there exists a considerable body of literature on behavioural topics based on very small sample sizes, and although large sample sizes are desirable whenever possible, these studies indicate that sound scientific investigations have been and can be conducted using small sample sizes as long as the necessary prudence is used in drawing conclusions based on the results obtained.

Captive versus wild studies.

Animals are creatures of the wild and if one wishes to learn about their behaviours and social lives one should study them in their natural environments. This statement reflects the ideal situation for obtaining knowledge about a particular species, but unfortunately the reality does not always live up to the ideal. In practice, there are all sorts of considerations that have to be taken into account when planning a research project, and there will always be some level of compromise between what is ideal and what is realistic. It is easy to plan an ideal project if practicalities are ignored. The most frequently encountered reasons for departing from the ideal are financial, logistic and time constraints. The species chosen for the study and its habitat will decide how extensive the compromises have to be.

In the present study compromises had to be made. The subject of the study i.e. the chemocommunication system of lions, tigers and leopards and its relation to their social systems, imposed its own constraints on the research design. In studying the scent marking behaviour of animals and their reactions to scent marks of conspecifics, it is important to be in continuous visual contact with the focal animals. Only one of the three study species can fulfil this requirement in the wild, namely the lion, which lives on the open African savannah. Both the tiger and the leopard are extremely evasive species which live predominantly in woodland or dense forest making it impossible to carry out direct observations of an animal for any length of time. The fact that the *Panthera* species are active mostly during the night in the wild would further complicate observations on scent mark depositions and responses. Financial and logistic problems involved in travelling between three continents to collect data as well as the limited time available for the field work imposes further constraints on the research design. Under these circumstances it was decided to carry out the present study on populations of captive animals rather than on wild ones.

The decision to work on captive animals imposed various limits as to the type of data which could be collected and also to the number of animals available for the study as mentioned in the paragraphs above. It did, however, have a number of important advantages over a wild study in that it is possible to observe a given animal continuously for very long periods and, in this way, to obtain very detailed data on its behaviour. Furthermore it became possible to subject the animals to a number of experimental situations, i.e. scent presentations which would have been difficult or impossible to carry out in the wild because the movement patterns of the animals are very unpredictable. A further advantage was the increased ease and accuracy with which scent samples could be collected, transported and stored. The collection of fresh scent marks is of the utmost importance for the chemical analysis. The samples require immediate freezing after collection in order to minimise the evaporation of high volatile compounds. This would be especially important in a hot tropical climate.

Lions are relatively common in zoo collections and because of their social nature, larger groups are usually available for study. Tigers and leopards are less common in zoos than lions and because both species are highly asocial, they are usually kept alone or, at most, in pairs. This means that the numbers of tigers and leopards available for study are lower than that of lions and the sample size of these species will therefore be smaller. Therefore this study has concentrated on collecting data on lions, but additional data on tigers and leopards will be presented.

The captive environment obviously imposes certain limitations on the animals. They are not allowed to choose their own macro-environment, although within the enclosure, whether small or large, they can choose their own micro-environment. Thus the "territory" that an animal inhabits is not defined by resources or competition with other animals but rather by the physical restraint of movement imposed by the peripheral fencing. As the nutritional needs of the animals are catered for "on a plate" their activity will be influenced by the pattern of the feeding regime. A given animal cannot choose which other individuals it would like to share the enclosure with, but it can choose which of its cohabitants to interact with, and what the nature of these interactions will be. All these factors will influence the way in which a given animal behaves and this influence has to be considered carefully when evaluating the results obtained from observations on zoo animals.

The scent marking behaviour of captive animals may also be affected by these environmental factors. I am not aware of any studies comparing the scent marking rate of a captive individual with those of free-living conspecifics for any species. The rate of scent

marking could be either positively or negatively influenced by captivity. The positive effect would be seen if an animal turned scent marking into a stereotypic action with a high frequency of repetition. The negative response would be seen if scent marking was triggered only by stimuli from foreign individuals, in which case the absence of such individuals would lead to a decline in the scent marking rate. Further complicating factors are that any such effect would probably be specific to the particular individual being studied and the specific circumstances under which that individual was being kept.

The occurrence of the different types of scent marks will be highly dependent on the physical environment of the animal. If the substratum is concrete, or other such hard surface, *Scrape/urination* is likely to be absent or, at least, much reduced in frequency. Similarly *Spraymarking* is dependent on suitable vertical objects and surfaces on which the animals can deposit this type of scent mark. The availability of suitable sites will also influence the number of scent posts used, and thereby the frequency of re-marking of any particular scent post. *Clawing* will only be seen in enclosures where trees or branches are present. Thus *Defaecation* and normal *Urination* are the only two types of marking which are unlikely to be seriously affected by the physical captive environment.

The enclosures used in this study probably provided the animals with reasonable opportunities for carrying out scent marking of all types. This impression is based on the fact that in all enclosures, the main part of the ground areas was covered with soft material in the form of gravel, earth or grass. Furthermore tree trunks and branches were present in all enclosures, as were other vertical structures suitable for *Spraymark* deposition. It would be impossible to try to evaluate the extent of the influence of the environmental factors on each individual in all of the enclosures, but the overall opportunities for marking behaviour of the animals studied were probably as good as those for wild animals.

There is an extensive literature on behavioural research carried out on captive animals. Early works in this area were published by Hediger in 1950 and 1955, and since then the area has expanded greatly. Examples of these types of study comprise both laboratory studies of smaller species of rodents e.g. mice (Wolff and Powell 1984, Sandnabba 1986, Gosling and McKay 1990), rats (Doty 1990, Natynczuk 1990), voles (Brinck and Hoffmeyer 1984) and carnivores e.g. mustelids and felids (de Boer 1977, Mellen 1993), as well as studies conducted in zoos and safariparks on larger species of ungulates e.g. Przewalski's horses, zebra and eland (Boyd 1988, Hogan *et al.* 1988, Kiley-Worthington 1978a,b, Andersen 1992a,b) and carnivores e.g. wolves (Asa *et al.* 1985, 1990) and some *Panthera* species (Asa 1993, Brahmachary *et al.* 1992). These studies have focused on a

number of issues ranging from sensory abilities, welfare and the animals' expression of natural and unnatural behaviours under captive conditions. Results from such studies have contributed to our knowledge of many of these species, and they can be used as a foundation for carrying out projects on the same species in the wild and thus focus this effort, which is usually much more costly both in time and money, in a much more efficient way.

Figure 2.1: Map of Denmark and Sweden indicating the primary study sites (**Copenhagen**, **Givskud** and **Knuthenborg**) and the location of the zoos which supplied additional scent samples for experiments and analysis (Aalborg, Odense, and Kolmaarten). (Sweden is not shown to same scale).



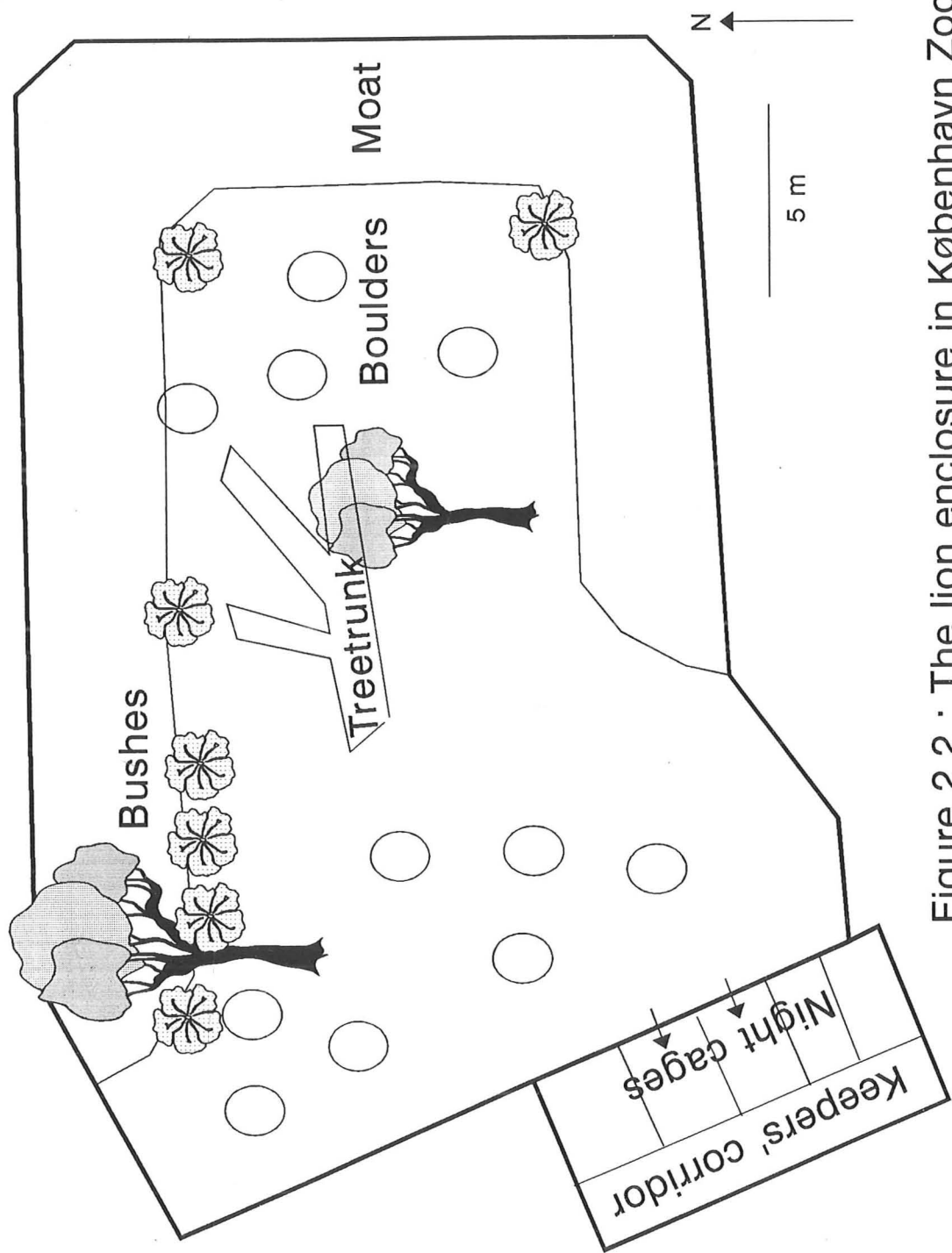


Figure 2.2 : The lion enclosure in København Zoo.

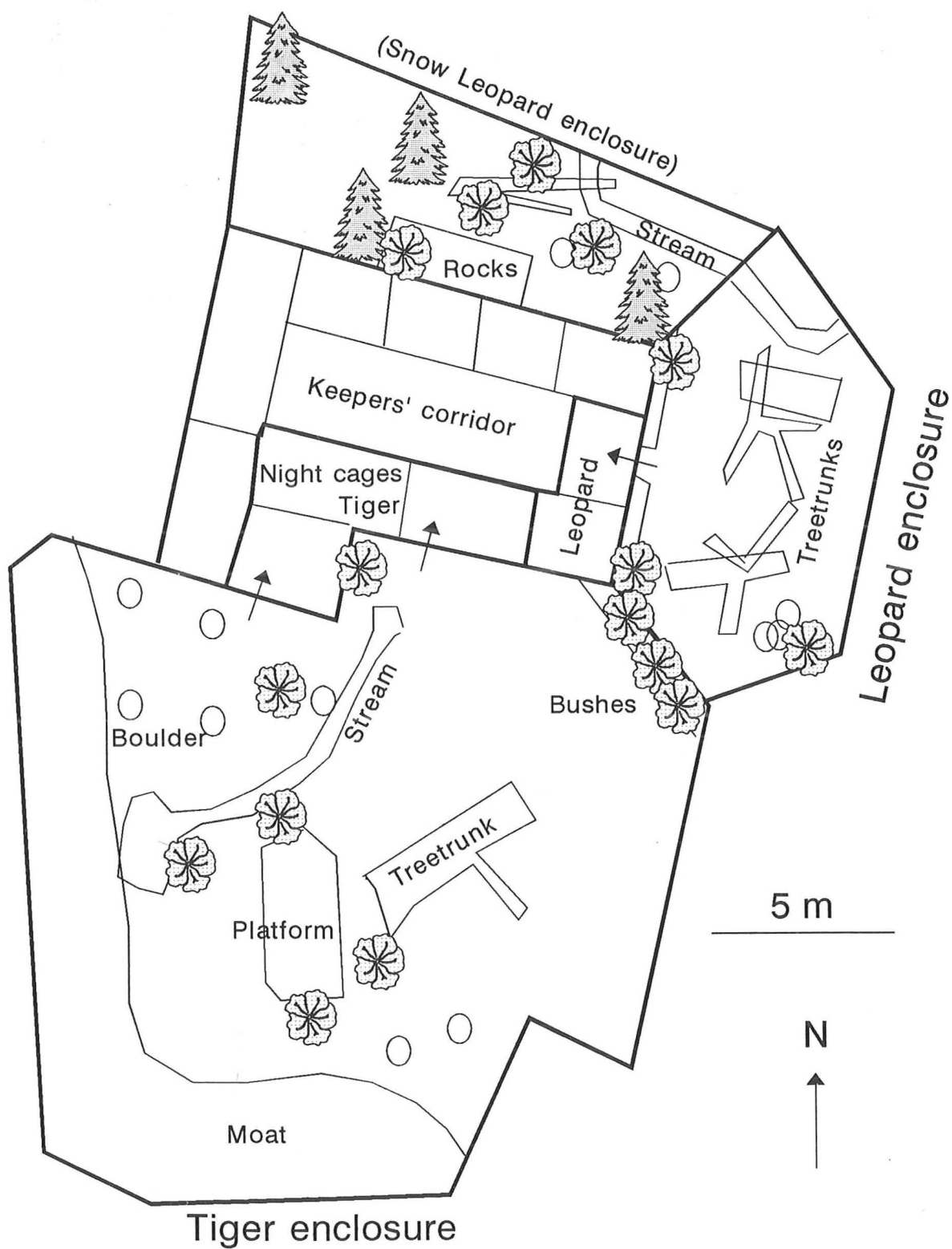


Figure 2.3 : The tiger and leopard enclosures in København Zoo.

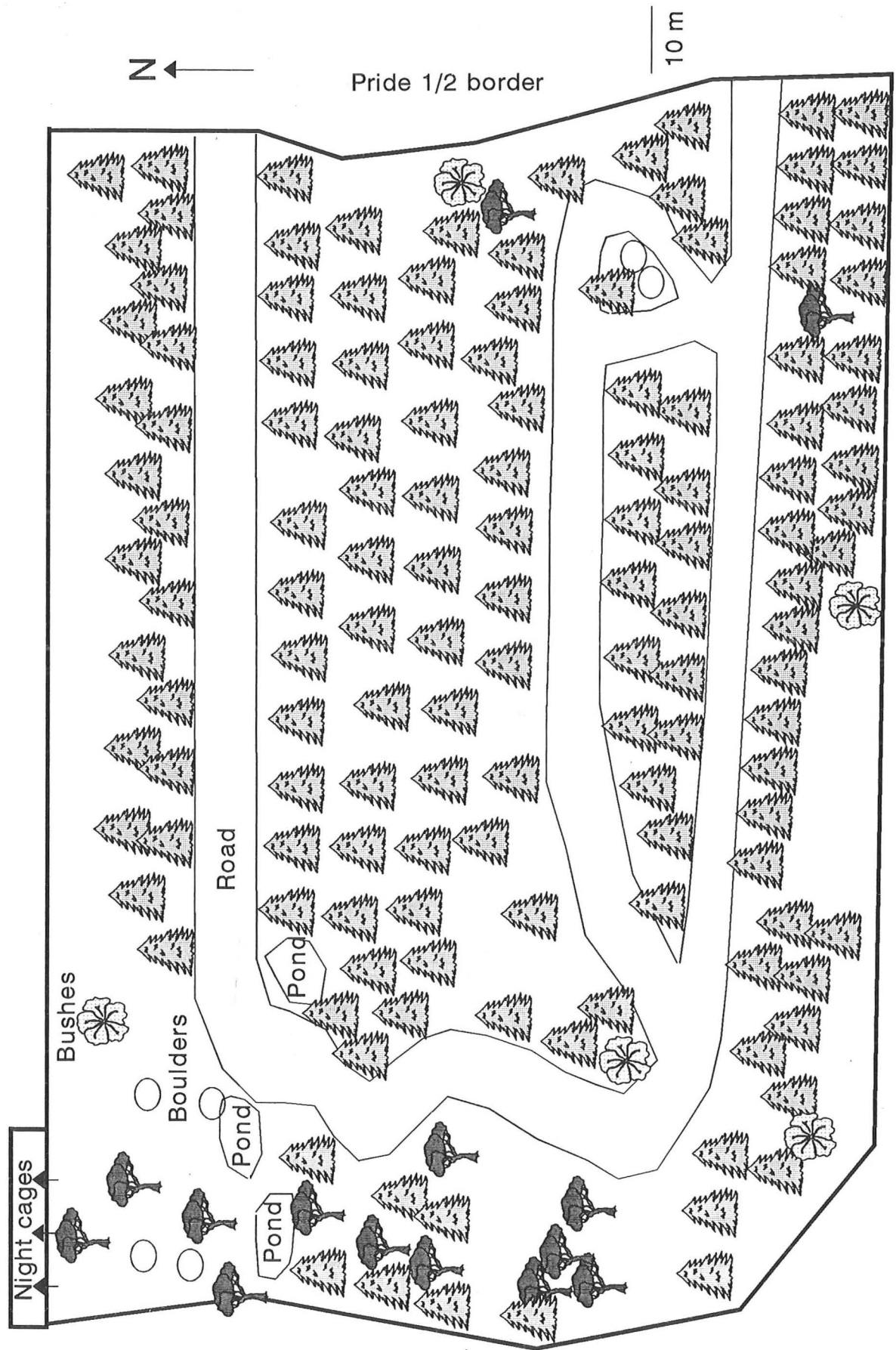


Figure 2.4 : The lion enclosure in Givskud.

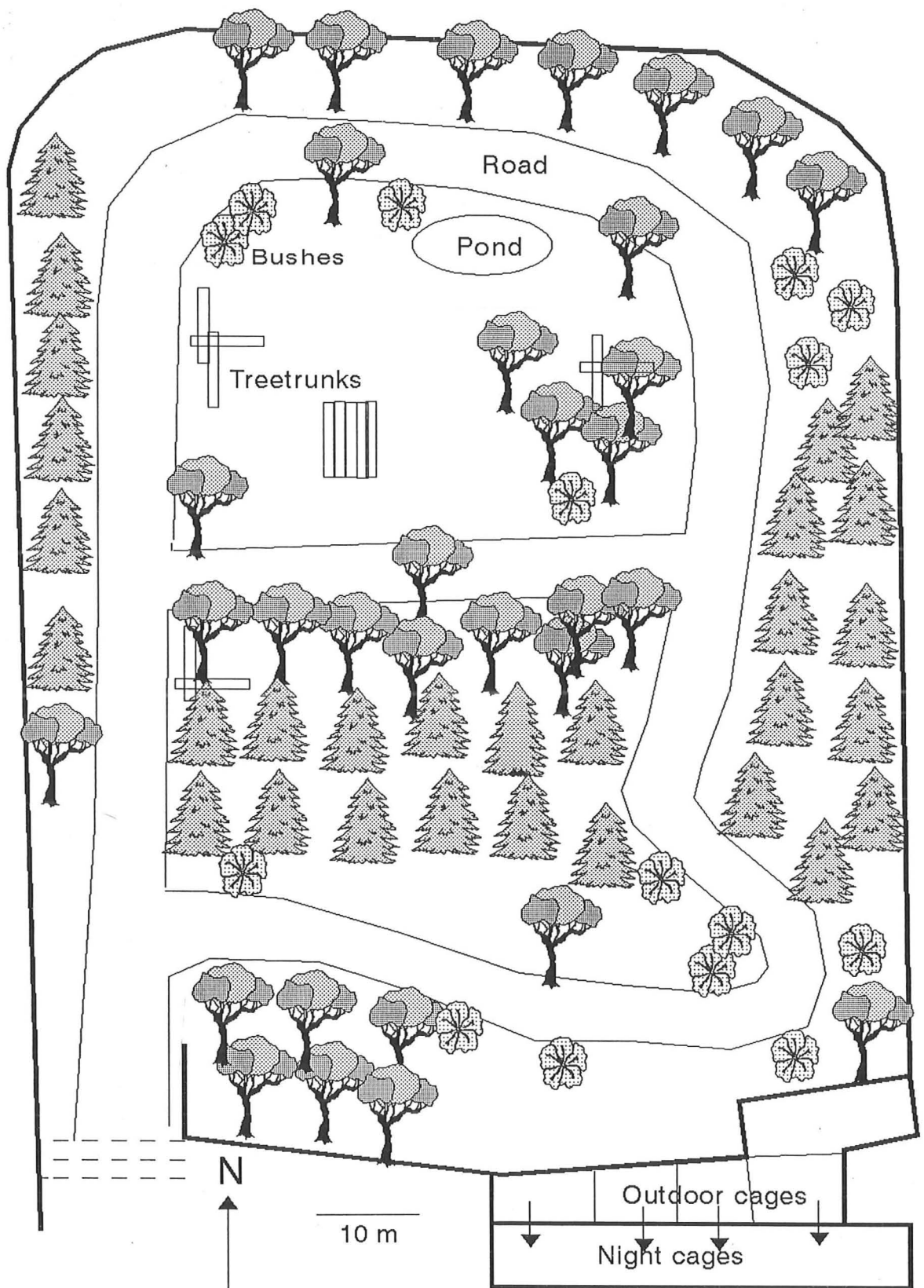


Figure 2.5 : The tiger enclosure in Knuthenborg.



CHAPTER 3. THE LION, *PANTHERA LEO*.

In this chapter the results on activity, social behaviour, scent marking behaviour and the chemical analysis of lion scent marks are presented. The first two sections presents baseline data which are used in evaluating whether the focal lion groups in this study are behaving similarly to wild lions, or whether any deviations in activity or behaviour occurs which may influence the data recorded on scent marking behaviour. The last two sections give data on scent marking behaviour and the chemical composition of the scent mark, and these results are used to test the hypothesis outlined in the Introduction chapter. Each section is followed by a discussion of the results obtained in this study in relation to other studies of lions both wild and captive. The figures and tables referred to in the text can be found on pages 81 to 104.

3.1 Activity.

The results presented on activity of the lions are part of the baseline data which are used to evaluate if the focal groups of lions in this study behave in a way similar to that of wild lions, and therefore can be expected to show corresponding levels of scent marking behaviour. Please refer to page 30 for a definition of the activity categories.

Results.

The main activity for all lions in both Copenhagen (KBH) and Givskud (GIV) was *Lying* (mean 81.6% \pm 10.9), followed by *Moving* (mean 10.4% \pm 7.3), *Standing* (mean 3.5% \pm 2.3), *Other* (mean 3.5% \pm 5.7) and finally *Sitting* (mean 0.8% \pm 1.2) (**Figure 3.1**). One of the male lions, Polle, exhibited a much higher level of activity (*Moving*) than all the rest of the individuals. There was a significant overall difference between the activities of the lion sex/age groups ($\chi^2=126.6$, DF=8,

$P < 0.001$). In **Figure 3.2** the contribution of each cell in the Chi-square test to the overall Chi-square value is shown for each of the sex/age groups in combination with the direction of the deviation. The two main differences between the sex/age groups, and therefore main contributors to the overall Chi-square value, are the higher levels of male *Standing* and of the combined cub groups doing *Other* activities. Other notable differences are the low level of cubs *Moving* and *Standing*, as well as the high level of female *Moving* and low level of female engaged in *Other* activities.

Discussion.

As pointed out in the Introduction chapter the main period of activity for wild lions is between dusk and dawn when they hunt for food (Schaller 1972, Bertram 1978, Rudnai 1979). As the captive lions in the present study was mainly confined to the night cages during nighttime they had to incorporate this restriction of access to the outdoor enclosures into their activity pattern. This enforced shift could possibly lead to changes in the overall activity budget of the lions. In the following paragraphs it will be made clear if such a shift was indeed observed.

The overall picture of lion activity is fairly homogeneous despite the significant difference found between the sex/age groups. The high level of *Standing* for the male lions compared to the other two sex/age groups could indicate a higher level of alertness in the males. Lion cubs engage in frequent playing, and this behaviour is the main reason for the high level of *Other* activity found in the cubs (see next section on social behaviours). No specific reason for the high level of *Moving* for the male lion Polle has been found.

The activity pattern for the two lion populations studied compares very well with those reported for wild lions and for other captive lion populations. *Lying* (or inactivity) is always the main category with figures for wild lions ranging from 75% to 88% of the activity budget. *Moving* accounts for 5-16% of the total budget (Rudnai 1979; Van Orsdol *et al.* 1985; Orford *et al.* 1988; Hanby *et al.* 1995). Exner (1995) reports figures of 78% for *Lying* and 16% for *Moving* from her study on captive lions. Even though differences were evident in the activity of the individual lions studied here all values falls within or are close to the limits of the above mentioned studies. On these grounds it is reasonable to conclude that there is no indication that unusual levels or natures of activity will have

influenced the results on scent marking behaviour presented later in this chapter in either a positive or a negative way.

3.2 Social behaviours.

The results presented on lion social behaviour are also part of the baseline data used in the evaluation of any effects which abnormal behaviours might have on the scent marking of the animals. Please refer to page 30 for a definition of the social behaviour categories.

Results.

The first step in the analysis of the social interactions of the individuals in the two lion prides was to draw a figure showing the direction of interactions between the individuals (qualitative analysis). The direction is defined by an animal performing a social behaviour (the "Actor") towards another animal (the "Target"). These are presented in **Figure 3.3** and **Figure 3.4** for the KBH and the GIV prides respectively. A number of common trends were seen for both prides.

Headrubbing: In each zoo the males were the main targets for *Headrubbing*, but the adult females were also targeted in a substantial proportion of the interactions, particularly in KBH. Cubs were mostly seen as actors of this behaviour, though in KBH they were also targeted for *Headrubbing*.

Bodyrubbing: The males in both prides were almost exclusively targets of *Bodyrubbing*, which was performed by both adult females as well as by cubs.

Allogrooming: The main focal point for *Allogrooming* was the cub groups, both as actors as well as targets. It was however also seen between the adult individuals, with females, especially Elsa in KBH and Flora in GIV, being the main targets.

Playing: Cub-cub interactions were the main contributor to the *Playing* category, but cub acting towards female and female acting towards another female were also

prominent. Males were targeted more for *Playing* behaviour than they were seen as actors. This was especially pronounced in the GIV pride.

Investigation: Although observed in most individuals, *Investigation*, which occurred in both prides, was skewed towards two main groups. The first group was the adult males, and in GIV the biggest of the two males, Flop, was particularly active. The second group was those adult females which were mated by the males at some point during the observation period (see category *Mating* in the Figures and below).

Mild Aggression: In both prides *Mild Aggression* was spread throughout the group, but particular adults seemed to be targeted more for this behaviour than others. This was evident in GIV in which Linda, Lene and the two males were targeted more often than the other adults. The cubs were also major targets.

Serious Aggression: *Serious Aggression* was observed only infrequently in both prides. In KBH it was almost exclusively confined to the adults, whereas in GIV the cubs and the two males were the main targets.

Mating: Real *Mating* was observed between the adult males and any female who happened to come into oestrus during the observation period. A variation of actual mating behaviour, here termed Mock mating, was also observed in both lion prides. Mock mating took the form of one animal of either sex mounting another animal, usually of the same sex, which was lying down either on its side or sternum, and then the mounting animal would perform a series of pelvic thrusts. Mock mating was seen between the females, between the females and the cubs and within the cub group. It was not observed for the adult males.

The second step in the analysis of the social behaviours of the two focal lion groups was a quantitative study of the social repertoire of each of the individual adult lions and the cub groups. These results are presented in **Figure 3.5**. It is evident that there are quite large differences between the individuals with regards to their behavioural repertoire, but some general differences between the sex/age groups are also found. A Chi-square test of the sex/age repertoires show a very significant difference between the groups ($\chi^2 = 864.1$, $DF = 10$, $P < 0.001$).

In **Figure 3.6** the relative contribution of each to the deviations to the total Chi-square value is shown for the three sex/age groups combined with the direction of the deviations. It is clear that the high level of male *Investigation* is the most prominent contributor to the total Chi-square value, followed by the high level of cubs *Play*, the low level of male *Play*, the high level of male *Mild-Aggression* and the low level of cubs *Mild-Aggression*. More minor contributors are the low level of cubs *Investigation* and female *Play* as well as the high level of female *Headrubbing*.

The third and final step in the analysis of the social behaviours within the two lion groups is an analysis of the relationship between sex/age groups and the amount of *Non-Aggressive* and *Aggressive* behaviours performed and received. The results were produced by adding the number of interactions in the *Non-Aggressive* behavioural categories and comparing them to the number of interactions in the two *Aggressive* behavioural categories. The *Non-Aggressive* categories were *Headrubbing*, *Bodyrubbing*, *Allogrooming*, *Playing* and *Investigating*, and the *Aggressive* categories were *Mild-Aggression* and *Serious-Aggression*. **Figure 3.7** shows the results from the KBH group, and **Figure 3.8** from the GIV group.

There is no difference in the way that the two males Willy in KBH and Flop in GIV were treated by females and cubs ($\chi^2=0.04$, DF=1, $P>0.05$; $\chi^2=5.36$, DF=2, $P>0.05$ respectively). They both received far more *Non-Aggressive* than *Aggressive* behaviours. The second male from GIV, Nero, was treated significantly different by the three sex/age groups ($\chi^2=36.55$, DF=2, $P<0.001$), and in this respect he was different from the two other males. He received more *Aggressive* than *Non-Aggressive* behaviours from the females. Females in KBH were treated significantly different by the three sex/age groups ($\chi^2=39.35$, DF=2, $P<0.001$) in that male *Aggressive* behaviours were high compared to *Non-Aggressive*, though the latter still prevailed. Similarly in GIV the females received a high level of *Aggression* in relation to *Non-Aggression* from the males when compared with the other two groups ($\chi^2=155.24$, DF=3, $P<0.001$), and especially from Flop in which *Aggression* was much higher than *Non-Aggression*. The cubs in KBH received much more *Aggression* than *Non-Aggression* from the male, and the overall sex/age group difference was significant ($\chi^2=154.41$, DF=2, $P<0.001$). The same was seen in GIV where the cubs also received more *Aggression* than *Non-Aggression* from the males, and even the female *Aggression* level was quite high though still lower than the *Non-*

Aggression level. Again the overall sex/age group difference was significant ($\chi^2=229.20$, DF=3, $P<0.001$).

Discussion.

The social environment of the lions closely reflects that found among wild lions, except for the fact that the adult males were kept together with the rest of the pride at all times. In GIV the size of the enclosure coupled with lush vegetation made it possible for the pride, and therefore also the adult males, to split up into sub-prides without visual contact. Each pride had one or more pride males, a number of adult female lions and their offspring. In the two study prides cubs of various ages were present at all times. Few detailed studies of social interactions between the individuals of wild living lion prides have been published, but some observations have been reported.

Headrubbing: Schaller (1972) and Bertram (1978) both noted that *Headrubbing* was a very common form of greeting among lions. They found that adult males mostly performed it among themselves, whereas they were frequently targeted for *Headrubbing* by both adult females and cubs. It was performed frequently between the adult females of a pride, and the cubs were usually more often actors than targets. This corresponds well with what was seen in the KBH and GIV study prides. Willy, the male in KBH, performed more *Headrubbing* towards females and cubs than did the two males Flop and Nero in GIV. This may be due to the fact that Willy was the only male in the group thus lacking male partners with whom to indulge in this activity, and also that the group was very small.

Bodyrubbing: No qualitative accounts for *Bodyrubbing* has been found for wild lions, but from the two study prides it was clear that this behaviour is almost exclusively performed towards the adult males by both adult females and cubs. It is sometimes seen as part of a greeting where it usually precedes *Headrubbing*. It is also performed as a part of the female mating behaviour, in which the female slides along the body of the male and then invites him to copulate by crouching in front of him in the normal mating posture.

Aggression level. Again the overall sex/age group difference was significant ($\chi^2=229.20$, $DF=3$, $P<0.001$).

Discussion.

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Allogrooming: *Allogrooming* is usually seen as having a social function (tie-bonding between individuals and appeasement) as well as a cleaning function. Observations on *Allogrooming* was made by Schaller (1972) and Bertram (1978). They found that the adult males rarely participated in this activity. Furthermore they noted that older cubs were actors of this behaviour at rates equivalent to those of adult females, whereas young cubs were mainly targets. Again this picture fits rather well with what was found for the two study prides. However the only individual which never either performed or received this behaviour during my observations was not an adult male but an adult female (Fifi) in the GIV pride.

Playing: *Playing* is widespread throughout the prides with the exception of the adult males who seldom participate in this activity. This was noted by Schaller (1972) and Bertram (1978), and the results from this study conforms with this general pattern.

Investigating: No qualitative reports on *Investigating* behaviour among wild lions are available, but from this study it appears that though it occurs throughout the pride, it is mainly shown by the adult males towards females which are in oestrus and vice versa. Its function is therefore probably to check the reproductive status of other individual lions.

Mating: This was of course also the case with real *Mating* behaviour, but mock matings were seen in all sex/age groups of the two prides. Mock mating has also been documented in wild lions. Schaller (1972) observed this behaviour among adult males, and he referred to it as "Homosexual" behaviour. He does not state whether it occurred among females or cubs. The function of Mock mating, if any, remains an open question.

Aggression in the form of threats and intention movements (*Mild Aggression*) were widespread in both prides, but actual strikes and bites (*Serious Aggression*) occurred much less frequently. This is also the case among wild lions (Schaller 1972; Bertram 1978). Both the adult males and the cubs were the main actors as well as targets for these behaviours, but one of the females, Linda in GIV, also featured prominently as both actor and target. This picture does not conform with Schenkel's observation of the lions of Nairobi National Park, that when young cubs were present in a pride "all the social

relations seem to be impregnated with tenderness and fondness and there is practically no room for social conflict and tension" (Schenkel 1966a). Whether this difference might be due to the captive conditions, or whether Schenkel's picture was a bit too "rosy" remains to be seen. It is possible that the restrictions in movement imposed by the captive conditions can lead to increased aggression because of the lack of escape possibilities when conflicts arise.

The social repertoire of each of the individuals in a lion pride is a dynamic entity which will depend on the relationship of the individual with the other members of the pride and which will be influenced by the sex, age and reproductive status of the individual. The previous experience of each individual will also be important in the way it acts towards the other group members. These facts are well reflected in the results obtained in this study. Every lion had a unique social repertoire, though some of the females had repertoires which were quite similar to each other. The two cub groups in KBH also had similar repertoires.

No studies from the wild have been found which gives figures for the social repertoire of each of the pride members, so it is difficult to put the results of this study into perspective. On a group level Hanby *et al.* (1995) did provide some figures for two lion populations, one in the Ngorongoro Crater and one in the Serengeti. They found that the social activity of each group was influenced by the environmental conditions in that the members of the lion prides were more socially active during a good season (measured in prey availability) than during a poor season. The levels of Playing, Affiliative and Hostile behaviours which they observed are very similar to the ranges found in this study. Exner (1995) noted for her captive lions that of the social interactions she observed *Headrubbing* ("Kopfreiben") and *Allogrooming* ("Lecken") made up 39%, *Playing* ("Spielen") 29%, *Mild Aggression* ("Drohen") 10% and *Serious Aggression* ("Angreifen") 4%. She did not, however, include information on the sex/age composition of her study groups, so it is difficult to compare the results. Suffice to say that they fall within the ranges observed in this study.

The ratio of Non-Aggressive and Aggressive social interactions observed between the different sex/age groups at the two locations were very similar, although some differences were also evident. The general picture is one of a sliding scale of Non-Aggression in relation to Aggression for the different sex/age groups, with the scale sliding towards Non-Aggression received by the strongest of the groups (males) from the other groups,

and towards Aggression received by the weakest group (cubs) from the other groups. No comparable results exists for wild lion prides.

It thus appears that both with regard to the general activity level and to the amount and nature of the social interactions within the two lion prides, these results are quite similar to those one would expect to find for wild lions. There is therefore no reason to assume that any of these factors should have any distorting influence on the scent marking data presented in the following sections.

3.3 Scent marking.

This section presents data on the scent marking behaviour of the focal lion groups both undisturbed and in experimental situations. These observations were carried out in order to test Hypothesis 1 by looking for differences in the marking behaviour of the sexes, and to quantify any such differences found. The results of the scent presentation experiments performed on the animals are also presented. These experiments were carried out to test Hypotheses 4, 6, 7 and 8 by investigating the animals' ability to distinguish between individual scent marks and between different categories of scent marks.

In all experiments distilled water was used as a neutral control mark. As no reaction was ever observed towards the water marks they have been excluded from the figures and tables in the results section below.

In the previous section it was established that the captive conditions had little apparent effect on the activity and social behaviours of the lions. It thus appears that, at least with regards to these two aspects, no corrective modifications have to be made when investigating the scent marking behaviour of the animals.

Defaecation: In both lion prides *Defaecation* was observed only infrequently. Faeces were deposited with no apparent significance being placed on the way of deposition or the location. It was never observed in combination with scrapes. Therefore *Defaecation* is excluded from the following presentation of results.

Results.

Scent marking repertoire.

Males.

Five main types of scent marking were observed for the lions, namely *Spraymarking*, *Clawing*, *Scrape/urination*, *Chinrubbing* and *Urination*. The percentage distribution of these five mark types for male lions are presented in **Figure 3.9**. From this figure it is clear that *Scrape/urination* was the mark type which varied least between males. *Scrape/urination* and *Spraymarking* were the two main mark types for the males in general, whereas *Urination*, and especially *Clawing* and *Chinrubbing* occurred irregularly. There was an overall significant difference in the percentage distribution of scent marks for the five males ($\chi^2=260.35$, $DF=16$, $P<0.001$). The main contributor to this difference was the male Polle in GIV which had a much lower percentage of *Spraymarking* and higher percentages of *Clawing* and *Urination* than those seen in the other males. The KBH male Willy had a higher percentage of *Chinrubbing* than the others.

Females.

In **Figure 3.10** corresponding data for the female lions are presented. The three main types of marking was *Spraymarking*, *Scrape/urination* and *Urination*, whereas *Clawing* and *Chinrubbing* occurred irregularly. Overall there are three main patterns, one with a high percentage of *Spraymarking* (Fiona, Lene, Lillian), another with a low percentage of *Spraymarking* (Linda, Fifi, Elsa), and the third one completely without *Spraymarking* (Flora, Fie, Frida, Nanna). But even within each of these three groups there is considerable variation between individuals. The overall difference between the females was significantly different ($\chi^2=772.25$, $DF=36$, $P<0.001$). Almost half of the total Chi-square value is contributed by the two KBH males Nanna, with high *Clawing* and low *Scrape/urination* percentages, and Elsa, with high *Chinrubbing* and low *Scrape/urination* percentages.

Comparison between sex groups.

Because of the big variation within each sex group one has to be careful when looking for overall differences. A 2x5-table χ^2 -test show a significant difference between the mark distribution of the sexes ($\chi^2=146.43$, $DF=4$, $P<0.001$), with the main contributors being the low level of male *Urination* compared to the females high level, and the high level of male *Spraymarking* compared to the low female level. However, if one test the mark distribution of the three females with the high *Spraymarking* level (Fiona, Lene, Lillian) against the males' distribution, the result turns out to be non-significant ($\chi^2=9.01$, $DF=4$, $P>0.05$).

A closer comparison between the sexes of the mean percentage distribution of *Spraymarking* (Figure 3.11) and *Scrape/urination* (Figure 3.12) revealed the following. There was a very strong tendency for males to have a higher mean percentage distribution of *Spraymarking* than females, though the difference was not significant ($t=2.01$, $DF=13$, $P=0.066$). The mean percentage distribution of *Scrape/urination* for males and females were almost identical, despite the fact that two females did not perform this behaviour at all ($t=0.027$, $DF=13$, $P=0.9787$).

Scent marking rates.

Males.

The rates of scent mark deposition of the different mark types for the male lions are depicted in Figure 3.13. The most frequently deposited mark type for four of the five males was *Spraymarking*. Only Polle had *Scrape/urination* as the most frequently deposited mark type. The second most frequently deposited mark was *Scrape/urination*.

Females.

Figure 3.14 show corresponding marking rates for the female lions. Two of the females (Lene and Lillian) had rates of *Spraymarking* and *Scrape/urination* which were much higher than the rest. The same three subgroups which were evident for the percentage distribution of scent marks, were also found for the marking rates. One group (Fiona, Lene and Lillian) being dominated by high rates of *Spraymarking*,

another group (Linda, Fifi and Frida) being dominated by high rates of *Scrape/urination* coupled with low rates of *Spraymarking*, and the third group (Flora, Fie, Nanna and Elsa) having high rates of *Urination* coupled with no *Spraymarking*. Note that Frida and Elsa have changed places, otherwise the group compositions are the same as for the percentage distribution.

Comparison between sex groups.

A look at Figure 3.13 and 3.14 (note the difference in scale of the Y-axis) shows that the female marking rates were generally well below those of the males. For all mark types the rates varied considerably within each sex group, and this makes a comparison between the sex groups more difficult and the result less clear. In **Figure 3.15** the *Spraymarking* rates for the two sexes are depicted. It is clear that there was quite a big difference in the mean values with males having much higher rates than females, but because of the large variation within each sex group, the t-test comes out just short of being significant (unequal variance, $t=2.618$, $DF=4,1$, $P=0.058$). **Figure 3.16** shows a comparison of the *Scrape/urination* marking rates of the two sexes. Again there was a clear difference in the mean values with males having the highest rates, and this time the variation within the sex groups is less than that for *Spraymarking*. This analysis shows that a significant difference between the male rate and the female rate existed ($t=7.152$, $DF=13$, $P<0.001$).

Context of scent marking.

In GIV the context of the two most common marking types, *Spraymarking* and *Scrape/urination*, were allocated to one of the following three categories :

Territorial: those markings which were directly linked to territorial behaviour.

Social: those markings which were directly linked to social interactions.

Unknown: those markings which did not show any obvious link to either of the two previous categories.

These results are presented in **Figure 3.17**. From the figure it is evident that around 42% of all male markings could not be directly related to either territorial or social behaviours, whereas the corresponding number for the females was 65%. If only those markings which could be directly related to territorial or social contexts are considered the following patterns emerge (**Table 3.1**). For the males there was a significant difference in the ratio of territorial-related and social-related markings for the two marking types with *Spraymarking* being predominantly seen in the territorial as opposed to social context and *Scrape/urination* showing an equal percentage in the two situations (G-test: $p < 0.001$). No significant difference was found for the females where both mark types were seen predominantly in the territorial context. The two sex groups had a similar, and therefore non-significant, pattern of territorial-related and social-related *Spraymarkings*, but for *Scrape/urination* markings there was a significant difference between the sexes (G-test: $0.001 < p < 0.01$), with females performing more of this behaviour in territorial contexts and less in the social context than males.

There was a higher concentration of markings along the common border with Pride 2 than throughout the rest of the enclosure. The second highest concentration was found along the outside wall of the night cages.

Investigation of natural scent marks.

The investigation of the reactions of the pride members to natural scent marks deposited by one of the other pride members was complicated by the fact that the exact location of the mark could not be memorized for long by the observer. In KBH only two reactions were observed and these were directed towards a *Urination* mark deposited by one of the adult females. The reacting animals were the male and the other adult female. In GIV 17 reactions could be classified accurately. Of these 11 were reactions provoked by *Spraymarkings* (eight towards male marks and three towards female marks), and six were directed towards *Scrape/urinations* (one towards male marks and five towards female marks). There appear to be a tendency towards investigations being directed predominantly towards male *Spraymarkings* and female *Scrape/urinations*, but the numbers are too small to perform any detailed analysis. At both locations the vast majority

of reactions to other scent marks was *Scenting* and *Flehmen* with *Licking* and *Overmarking* being observed very rarely.

Presentation and reaction to experimental scent marks.

The first set of results presented illustrates the qualitative nature of the lions' responses i.e. whether there were any differences in the proportion of the marks reacted to for the different categories of experimental marks. Data from the Indoor and Outdoor experiments in both KBH and GIV were combined to produce a total picture of the lions' responses and they are shown in **Figure 3.18**. The patterns are not significantly different for any of the three sex/age groups, but the general trends which can be found are the males' positive or neutral response to Female oestrous marks and mostly positive response to Tiger marks. The females are very mixed in their reactions with no clear preferences. The cubs show negative responses to Female or Female oestrous marks and a positive response to Tiger marks. The data were also depicted separately to see if the response was the same in the four different settings. These are depicted in **Figure 3.19** (KBH Indoor), **Figure 3.20** (KBH Outdoor), **Figure 3.21** (GIV Indoor) and **Figure 3.22** (GIV Outdoor). As is obvious from the figures there is quite a lot of variation in the responsiveness of the lions to the experimental marks under the four conditions, and indeed none of the patterns for any of the sex/age groups under any of the four conditions comes out significant in a χ^2 -test. The general trends outlined above for Figure 3.18 are found again in the four figures.

The different circumstances under which the presentations took place had a major impact on the responsiveness of the lions. In the Indoor experiments in KBH 147 presentations took place and 106 of these marks were reacted to by one or more of the lions, giving a response of 72%. The Outdoor KBH experiments consisted of 171 mark presentations of which 93 were reacted to, resulting in a response of 54%. In GIV the response in the Indoor experiments was 43% (64 reactions to 150 presentations), and in the Outdoor experiments it was as low as 20% (26 reactions to 129 presentations).

When a lion reacts to a scent mark, whether natural or experimental, its reaction is characterised by certain types of behaviour. The first reaction will be to approach to mark and sniff it (*Scenting*). *Scenting* may be followed by either one of three additional behaviours or a combination of them. These are *Flehmen*, in which the animal uses its Jacobson's organ to investigate the scent, *Licking* of the scent mark, and *Overmarking* the scent mark with its own personal scent. The first two types of reactions are by far the most common and these were investigated in detail for the lions when they were investigating different types of experimental marks.

The frequency of *Scenting* reactions for lion males, females and cubs to different categories of experimental scent mark types are depicted in **Figure 3.23a,b and c** respectively. The frequency was calculated by dividing the number of *Scentings* observed by the number of mark presentations taking place in each situation. When comparing sex/age groups and scent mark categories the special statistical method developed by Jon Stene and described in Appendix 2 was used.

There were considerable differences in the *Scenting* frequencies between the four experimental series (KBH Outdoor and Indoor, and GIV Outdoor and Indoor) and therefore the data could not be pooled within each sex/age group. The two KBH series generally had higher frequencies than the two GIV series for all three sex/age groups.

Male lions (Figure 3.23a): There were no consistent significant differences in the males' reaction to the different scent mark types in the four experimental series. There was a general tendency for Lion female marks to receive less *Scenting* than Lion male marks. In the KBH Outdoor series Lion male marks and Tiger marks received significantly more *Scenting* than Leopard marks ($P=0.023$ and $P=0.036$ respectively)). For the other three series there were no significant differences between mark types.

Female lions (Figure 3.23b): No overall significant differences were found for scent mark types between the four experimental series. In the KBH Outdoor series Tiger marks received a significantly higher frequency of *Scenting* than Leopard marks ($P=0.005$), and in the GIV Outdoor series Tiger marks received significantly more *Scenting* than both Lion male ($P=0.007$) and Lion female marks ($P=0.001$).

Cub lions (Figure 3.23c): No overall significant differences were found for scent mark types between the four experimental series. Lion female oestrous marks generally received less *Scenting* than the other mark types and Tiger marks received more. In the KBH Outdoor series Tiger marks received significantly more *Scenting* than Lion female oestrous marks ($P=0.025$), and in the KBH Indoor series Tiger marks received more *Scenting* than Lion male marks ($P<0.001$), Lion female marks ($P=0.002$) and Lion female oestrous marks ($P=0.002$). Tiger marks were again the highest receivers of *Scenting* in the GIV Indoor series with a significantly higher level than both Lion male marks ($P=0.002$) and Lion female marks ($P=0.011$).

In Figure 3.24a,b,c and d the three sex/age groups are compared in their reactions to the different types of experimental marks in the four experimental series. The only general trend which is evident from the four figures is that the two adult groups tended to have higher *Scenting* frequencies than the cubs when investigating the experimental marks, and this was especially true for the three Lion scent marks.

KBH Outdoor (Figure 3.24a): The male lions showed a significantly higher frequency of *Scenting* towards Lion male marks than did either female or cub lions ($P=0.050$ and $P=0.031$ respectively), and they also showed a higher *Scenting* frequency towards Lion female oestrous marks than did the cubs ($P=0.032$).

KBH Indoor (Figure 3.24b): Only one significant difference was found between the sex/age groups in this series namely the higher *Scenting* frequency of female than cub lions towards Lion male marks ($P=0.031$).

GIV Outdoor (Figure 3.24c): The females had a significantly higher *Scenting* frequency than the cubs when investigating Tiger marks ($P=0.011$).

GIV Indoor (Figure 3.24d): No significant differences were found between the sex/age groups.

The frequencies of *Flehmen* showed towards the different types of experimental scent marks were investigated in a way similar to the *Scenting* frequencies. The results for each of the three sex/age groups are shown in **Figure 3.25a, b and c**. As for *Scenting* the results vary considerably between the four experimental series and the data could therefore not be pooled.

Male lions (Figure 3.25a): No significant differences in the frequency of *Flehmen* towards the different mark types was found for male lions in any of the four experimental series.

Female lions (Figure 3.25b): In the GIV Outdoor series Leopard marks received significantly more *Flehmen* than Lion male marks ($P=0.013$), and in the GIV Indoor series Tiger marks received more *Flehmen* than Lion female marks ($P=0.027$).

Cub lions (Figure 3.25c): There was a general tendency of higher *Flehmen* frequencies towards Tiger and Leopard marks than Lion marks. In both the KBH and GIV Indoor series Tiger marks received significantly more *Flehmen* than either Lion male ($P=0.001$ and $P=0.002$ respectively) or Lion female marks ($P=0.015$ and $P=0.003$ respectively).

In **Figure 3.26a,b,c and d** the three sex/age groups are compared in their *Flehmen* reactions to the different types of experimental marks in the four experimental series. The only general trend which is evident from the four figures is that Lion male and Lion female marks tended to provoke the lowest *Flehmen* frequencies at all four locations. There were no significant differences between any of the sex/age groups when investigating the different scent mark types at any of the locations.

The other two response categories which were investigated were observed only infrequently. *Licking* was very rare and *Overmarking* was mostly observed during the indoor experimental series. In KBH Willy was seen to overmark one of the experimental marks by *Spraymarking* on 28 occasions but no significant difference was found in the number of times each mark type was overmarked relative to the number of times it was presented (G-test: NS). In GIV the only individual which was observed to overmark any

of the experimental marks by *Spraymarking* was Flop. This happened on only four occasions, and no significant differences were found between the number of *Spraymarkings* provoked by each mark type and its relative number of presentations (G-test: NS).

Discrimination of scent from two individuals.

In the Indoor experiments the experimental marks were presented four at a time, in no particular order, in a row with equal distance between the marks. The presentation usually comprised three identical marks and one mark which differed from the others, either in the individual, the sex, or the reproductive state, or a combination of these factors. This made it possible to collect the following type of data which was used to test Hypothesis 4.

The investigation of the Odd mark was noted and compared with the investigation of the three Like marks. The position of the first Like mark investigated (Like 1) was noted, and each subsequent investigation of this particular mark was scored in the Like 1 category. Similarly the position of the second Like mark investigated was noted (Like 2), and again any subsequent investigation of this mark was scored in the Like 2 category. The same procedure was followed for the Like 3 category. A separate record was kept for each of the animals as they investigated the marks so that the position of the Like 1 mark for one animal was not necessarily the same as the position of the Like 1 mark of another animal. Finally all the Odd, Like 1, Like 2 and Like 3 scores were added up within each category, and the results are presented in **Figure 3.27** for the KBH lions and in **Figure 3.28** for the GIV lions. For the KBH group the Odd and the Like 1 mark were investigated with almost equal intensity (unequal variance, $t=0.79$, $DF=24.7$, $0.05 < P$), whereas Like 1 was investigated significantly more than Like 2 (unequal variance, $t=3.12$, $DF=24.7$, $P=0.005$), and Like 2 was investigated significantly more than Like 3 (unequal variance, $t=2.68$, $DF=28.8$, $P=0.012$). In GIV the Odd mark was investigated significantly more than Like 1 (unequal variance, $t=2.32$, $DF=14.2$, $P=0.036$). Similarly Like 1 was investigated significantly more than Like 2 (unequal variance, $t=2.49$, $DF=12.6$, $P=0.028$), and Like 2 significantly more than Like 3 (unequal variance, $t=3.14$, $DF=14.1$, $P=0.007$).

Discussion.

Each of the different scent mark types used by lions has its own individual characteristics with regard to visual conspicuousness and longevity.

The most visually conspicuous of the different types of scent marks are *Clawing* and *Scrape/urination*. Although the clawing of trees may leave scent from the interdigital glands, the main communicatory function of this type of mark, if any, is likely to be visual. No accounts were found of animals conducting a thorough olfactory investigation of a "pure" claw-mark, and neither do such marks provoke *Flehmen* on their own. *Scrape/urination* leaves a very distinct mark both visually and olfactorily. The scrape marks may attract the attention of a passing animal to the chemical information available at that site. By the physical act of scraping and making an earth mound, the surface area on which the scent mark is deposited is increased manifold. This would increase the rate of evaporation from the mark and thereby also decrease its olfactory longevity. Thus *Scrape/urination* marks will have a long visual lifetime but only a moderate olfactory one. Sometimes faeces are deposited on top of scrapes instead of urine. These would increase the olfactory lifetime of the mark because faeces decompose much more slowly than urine evaporates, but it might also change the message conveyed as the chemical composition of faeces is different from that of urine. The deposition of faeces may be accompanied by secretions from the anal sacs, and Banks *et al.* (1992) reported that the range of amines found in the anal sac secretion of the tiger closely resembles that present in tiger urine. This indicates that the message contained in the urine and the anal sac secretion of the tiger may be very similar, if not identical. This may also be the case for lion urine and faeces.

Spraymarking is predominantly an olfactory mark though some visual changes to the marked spot may be evident. Right after deposition the marked spot will be wet and this could attract the attention of animals in the immediate vicinity of the mark. Within a very short time, depending on the environmental conditions, the mark will lose its wet appearance and then the conspicuousness of the mark will depend on its chemical composition. Lion and leopard marks are not readily visible after they dry out, but tiger marks contain a substantial amount of lipids, which after a while, turn black and tar-like thus making the marked site visible again. This is particularly obvious at marking posts

which are used repeatedly by the animals. If the marks are deposited on dark surfaces they will be less obvious and therefore harder to detect visually.

Chinrubbing may leave both visual and olfactory signs, but its use is very infrequent and it is doubtful whether it plays a very important role in the communication of the species studied.

Apart from its olfactory signal, normal *Urination* leaves a damp patch on the surface. This disappears quickly, and the only thing left is the chemical signal with no visual attractants. *Defaecation* is both visual and olfactory, but if the faeces are not deposited in a conspicuous place, their deposition may serve a purely metabolic function. It may be that the faeces left on scrapes are contaminated with anal sac secretion whereas other faeces are not, but there are as yet no data to support this hypothesis.

Scent marking behaviour of wild lions has received relatively little scientific attention until now and the only results available are purely descriptive. Among the first accounts are those of Fiedler (1957) and Schenkel (1966b) who both noted that apart from normal eliminatory urination, the lion also sprays jets of urine backwards from a standing position with the tail lifted. This spraying is usually directed towards a bush or tree trunk and is performed more often by the males than by the females. In addition Schenkel (1966b) noted another form of marking behaviour in which the lion scrapes its hind-feet alternately on the ground and adds a few jets of urine on top of the resulting mound. Schaller (1972) and Bertram (1978) also recorded this latter form of scent marking. Schenkel (1966b) showed that *Scrape/urination* was used more frequently than *Spraymarking*, and this finding was supported by Schaller (1972), who found that the ratio of *Scrape/urination* to *Spraymarking* was roughly 3:1. All authors state that these two types of scent marking are used as part of the lion's territorial behaviour. Schaller noted two further situations in which scent marking was common, namely in connection with agonistic social interactions and when males accompany oestrous females. Further types of marking were clawing a tree trunk and rubbing the cheeks along the surface of an object or a bush. The role which these two latter types of marking plays in the lions' communication system is unclear. Clawing is said to carry information on the size of the marking animal but whether this information is subsequently used by a passing animal is doubtful. The communicatory function of the cheek gland secretion has not been studied. No quantitative accounts of scent marking by wild lions were found.

On the basis of the results presented in this chapter it is evident that males have a higher percentage of *Spraymarking* in their marking repertoire than females. Furthermore they differ with regards to the rate of scent marking in that males had a significantly higher rate of *Scrape/urination* and a higher rate of *Spraymarking* than females. Both these differences lend support to Hypothesis 1. As reported for wild lions males had higher rates of marking than the females, although two of the females in GIV (Lene and Lillian) came close to the rates of the males with the lowest marking rates (Polle and Nero). Whether this difference in marking rates for the females can be linked to their individual personalities and roles in the pride would be interesting to know. Heinsohn and Packer (1995) reported that females take on different roles when defending the pride territory against other female groups. When provoked with playback calls of foreign females, some pride females would always lead the approach towards the source of the sound whereas other females would always lag behind. It may be that the leading females are also more active in the marking of the group territory and that this would create a difference in the marking rates of the females.

No evidence was found to indicate that lions use faeces as part of their scent marking system. *Chinrubbing* was observed very infrequently and its function is not clear.

The use of scent marking by lions in territorial and social contexts has been noted by several authors (Schenkel 1966b; Schaller 1972; Bertram 1978). Evidence was found in the present study to suggest that the sexes might be different in the way they use scent marks. Female lions showed equal proportions of *Spraymarking* and *Scrape/urination* in territorial and social contexts with *Spraymarking* three to four times as high as *Scrape/urination*, but for the males a significant difference in this proportion was found. It appears that the males use *Spraymarking* and *Scrape/urination* with equal frequencies in social contexts, but show a preference for *Spraymarking* in territorial contexts. This was indicated by the fact that 75% of all markings ascribed to the territorial category were *Spraymarkings*. These results should be treated with some caution as a large percentage (43% for males, 63% for females) of the total number of marks observed could not be directly ascribed to either of the two categories by the observer. This does not mean that the unknown markings could not have been triggered by either territorial or social motivations, but just that the motivation was not obvious to the observer. Again no data are available for wild lions on which to base a comparison.

Little is known about the reaction of a lion to scent marks. The basic behaviour involved in investigation of a scent mark is described in terms of *Scenting*, *Flehmen*, *Licking* and *Overmarking*. It is generally assumed that lions are able to extract information about other lions from their scent marks, and that they use this information in territorial and reproductive contexts, but apart from these general assumptions no detailed information is available. The experimental work conducted in this study is an effort to rectify this situation by testing specific hypotheses (Hypotheses 4, 6, 7 and 8) relating to the lions' ability to discriminate between scent marks.

The question of individual recognition is a complicated one. When the term "recognition" is used, it implies that an animal is able to associate a particular smell with a particular animal. If an animal is presented with two scents and is able to distinguish between them, it does not necessarily demonstrate "recognition" but rather discrimination. Thus discrimination is a distinct ability, and rather than being equivalent to recognition, it is the basis for it. The literature on individual discrimination in mammals is substantial and has been reviewed by Brown (1979) and Halpin (1980, 1986). The vast majority of studies on this subject have provided evidence for discrimination rather than recognition.

The data presented in this thesis are concerned with the ability to discriminate between the scent marks of two animals rather than with an animal's ability to recognise another individual by its scent.

Is there any evidence from the experiments carried out in this study that lions are able to distinguish between different scent marks? It was indeed found that the lions were able to distinguish between two scent marks of different origin. This was shown in a habituation experiment where four scent marks, three identical and one different, were presented to the animals simultaneously. The time which the animals spent investigating the four marks differed significantly, with highest investigation of the Odd and Like1 marks, and the Like2 and Like3 marks largely being ignored. This result supports Hypothesis 4 which predicted that animals would be able to distinguish between the scent marks of two foreign conspecifics.

It seems likely that lions are able to carry the distinction further than merely a discrimination between two marks. Ideally they should be able to tell whether a given mark originates from a male or a female (Hypothesis 7), and whether or not the female is in estrous (Hypothesis 6). Are there any evidence from the data presented in this chapter that this is in fact the case? If considered from the point of view of preferential response to

a particular type of scent mark there are some indications that this is indeed the case. Male lions showed the highest overall preference for Female estrous marks, and this mark type ought to be the most interesting one for a male lion in a biological reproductive context, and this result is evidence in support of Hypothesis 7. The results of the three sex/age groups towards male and female marks show slight differences and this suggests that lions are also able to distinguish between these two categories, but this result is only a somewhat tentative support for Hypothesis 6. Both male and cub lions showed a preferential response to Tiger marks but they were both more or less indifferent to Leopard marks, suggesting that lions are also able to distinguish between marks from these two other *Panthera* species, supporting Hypothesis 8.

The overall response percentages for the four experimental situations differed markedly, and it is clear that the small enclosure in KBH resulted in much higher response percentages than the large enclosure in GIV. It is also evident that the indoor setting where the marks were presented on the wall of the night cages produced more responses from the lions than the outdoor presentations. It therefore appears that the most efficient method of performing a scent bioassay on lions is by presenting the marks in the indoor setting.

When investigating scent marks the most common types of behaviour were *Scenting* and *Flehmen*. Hypotheses 6, 7 and 8 predict that there should be chemical differences between the scent marks of males and females, females and oestrous females, and lion and other *Panthera* species. The chemical side of these predictions will be dealt with in section 3.4, but as a consequence of these predictions one should be able to detect differences in the behavioural response of the lions to the different categories of scent marks (Hypothesis 4). This difference could well be reflected in the investigative behaviour of the animals towards the marks, i.e. the number of *Scenting* and *Flehmen* shown. Were such differences found?

There were indeed differences found in the reaction of each sex/age group towards the different types of experimental scent marks, but unfortunately these differences were not consistent within the sex groups over the four experimental series. Seen in relation to the social organisation of lions one would have expected to see clearer differences between some of the mark types. Lion males compete with other males for the control of territories and female prides and they should therefore be interested in the presence of scent marks from foreign males. There is a pool of nomadic males which are not in control of prides but which roam over larger areas, singly or in groups, on the lookout for a suitable pride

which it/they might take over if it/they can evict the present pride male(s). Therefore male lions should be aware of the presence of other males in their proximity. In this way pride holders can follow and evict intruders more efficiently, and the nomadic males can detect when they are inside the territory of other males, and decide whether to avoid the area or move closer and challenge the resident male(s). This decision could be based on their own numbers in relation to the number of resident males which they can possibly detect from the identity of the scent marks present in the territory. A general tendency of high *Scenting* and *Flehmen* frequencies towards foreign lion male marks was not seen in the present study.

From a biologically point of view lion males should be more interested in marks from oestrous females than non-oestrous females. In this way pride holders can keep track of when the females in their pride(s) are in oestrous even if he/they are not in actual physical contact with the females in question. It could also be used by the nomadic males to monitor the possibly immediate gain in a pride takeover by the presence of estrous females. In two out of three of the experimental series the males showed higher *Scenting* and *Flehmen* frequencies towards marks from oestrous females, but the picture is not clear-cut from the present experiments.

The female lions showed a tendency of more *Flehmen* towards lion female oestrous marks than towards lion male or female marks. What biological meaning lies behind this difference, if any, is difficult to say, but it could be related to synchrony of estrous periods and the resulting communal rearing of young which is often seen in lion prides (Packer 1986, Packer and Pusey 1995).

Generally all sex/age groups showed higher levels of both *Scenting* and *Flehmen* towards marks from tigers than towards leopard marks. This indicates an ability to distinguish between these two species on the basis of their urine.

There is a number of factors which could have had an influence on the results of the scent presentations. Even though I took all precautions in trying to deposit the experimental marks in a way as close as possible to the way the lions normally deposit scent marks themselves and on objects similar to the ones on which they would normally mark themselves, it might still be possible that the experimental marks appeared "artificial" to the lions and that this would dampen or alter their reactions towards them. Even if the lions perceived the experimental marks as natural marks they might not need to investigate them in a way different from that in which they would investigate familiar

marks in order to extract the relevant information from them. If they do react to and extract information from the marks they may choose to ignore this information completely or they may react to it in a way which would be undetectable to the outside observer.

Whether or not the lions are able to remember a certain scent over a period of time and recognise that same scent when it encounters it again is still an open question.

3.4 Chemical analysis.

This section presents and discusses the results of the chemical analysis which were carried out on the lion urine samples collected. The data were collected in order to produce results which could be used in the evaluation of Hypothesis 5.

Results.

Chemical compounds identified in lion urine:

On the basis of the identification process of the mass spectra a list of 81 different compounds was produced, each of them being found in one or more of the samples analyzed. Of these 81 compounds 18 were also identified in the accompanying control samples of laboratory air, and a further five were also found in the control sawdust extractions. Thus 58 compounds can with certainty be said to originate from the lion urine whereas the other 23 might have had different origins. All 81 compounds are listed as **Table 3.2** and **Table 3.3** with indication of their suspected origin. In **Table 3.2** are also listed the number of samples analyzed for each individual and the total number of compounds identified for these individuals excluding those compounds which were also found in corresponding control samples of laboratory air and sawdust extraction. These latter compounds have been excluded from the following analysis. As can be seen from **Table 3.2** the samples from the two lion males Napoleon and KolmHan contained many fewer compounds than the rest of the lion samples. The samples from these two males were not collected by the author himself, but sent to the author by the keepers of the respective Zoos. Instructions were given by the author to the keepers on how to collect

and keep the samples, but it is not known to what degree these instructions were followed. Furthermore the samples were exposed to normal ambient temperatures during the period in the post, and these two factors may go some way in explaining the obvious difference in compound numbers. Due to this difference these two individuals are excluded from a few of the following analysis.

Of the compounds identified in the lion urine numbers 12, 16, 20, 23, 50 and 70 were specific to male lions, although they were not found in all samples from male lions. Compound numbers 2, 3, 18, 19, 22, 30, 56, 57, 65, 68 and 71 were found only in female samples, though again they were not all found in all female samples. The rest were found in one or more samples from both sexes.

From the data in Table 3.2 an Average Linkage Cluster analysis was performed to clarify any underlying trends in the relatedness of compound composition for the different lions. The resulting dendrogram is shown in **Figure 3.29**. Three main clusters are seen but with no obvious separation between males and females.

Overlap in compound composition within and between the sex groups:

An overlap between two individuals or sex groups is calculated as the number of compounds common to both individuals or groups divided by the total numbers of compounds for each of the individuals or groups. If for instance a male and a female have 10 compounds in common and the male has a total of 20 compounds present in his urine, the overlap from the males point of view will be 50%. If the female has a total of only 15 compounds present in her urine, the overlap from the females point of view will be 67%. By comparing all male and female lions in this way total mean values for the Male-Female and the Female-Male overlap can be calculated. The same method is used for the calculations within each of the sex groups.

Figure 3.30 depicts the difference in compound overlap between the sexes (in this calculation the data from Napoleon and KolmHan are left out). A significant difference was found ($t=4.22$, $df=43$, $p<0.001$) between the mean overlap within the male group ($N=6$, $mean=79.7$, $SE=4.0$) and the mean overlap between males and females ($N=39$, $mean=59.9$, $SE=1.7$). No significant difference was found ($t=1.78$, $df=193$, $p=0.076$) for the corresponding comparison between the mean female overlap ($N=156$,

mean=66.5, SE=1.3) and the mean overlap between females and males (N=39, mean=71.6, SE=2.6).

Figure 3.31 show the mean overlap in compound composition for all different samples from the same individual ("within overlap") compared to the overlap of each individual with the other individuals of the same sex ("between overlap"). A t-test was used to analyze these data for significant differences. From the Figure it is evident that two of the males (Polle and Flop) and one of the females (Natasha) had a lower "within overlap" than "between overlap", the rest of the individuals all had a higher "within overlap" than "between overlap". In the male group only KolmHan had a significantly higher "within overlap" than "between overlap" ($t=4.82$, $DF=8$, $P=0.001$). For the females six out of eleven had significantly higher "within overlap" than "between overlap", namely Linda ($t=3.23$, $DF=16$, $P=0.005$), Fifi ($t=7.23$, $DF=22$, $P<0.001$), Lene ($t=5.81$, $DF=16$, $P<0.001$), Gurli ($t=5.37$, $DF=16$, $P<0.001$), Lilly ($t=2.79$, $DF=22$, $P=0.013$) and Nina ($t=2.26$, $DF=22$, $P=0.034$).

Differences in absolute and relative concentration of common compounds between the sexes.

A number of the compounds listed in Table 3.2 was found to be present in almost all individuals of both sexes. These compounds were numbers 4, 10, 26, 31, 37, 51 and 62, excluding the compounds which were also found in the two types of control samples. The absolute concentration (TIC) of each of these compounds was measured as the areas underneath each of the peaks on the respective chromatograms. The absolute concentration of each of the seven compounds for the two sexes are shown in **Figure 3.32**. A significant difference in concentration is seen between the sexes for compound 10 (2-butanone) with males having a much higher concentration than females (unequal variance, $t=2.50$, $DF=18.1$, $P=0.022$). None of the other compounds was found to have a significant difference in concentration between the sexes.

The mean percentage concentration of each of the seven common compounds were also calculated for male and female lions. The results are shown in **Figure 3.33**. Of these common compounds females were found to have a significantly higher mean percentage concentration of compound 4 (acetone) than did males ($t=2.32$, $DF=27$, $P=0.028$).

They also had higher mean percentage concentration of compounds 26 (1-pentene) and 62 (diethylbenzene) than males but not significantly so. Males were found to have higher mean percentage concentration of compound 10 (2-butanone) than did females though again not significantly so. For the rest of the common compounds there were no major differences in the concentration levels between the sexes.

Discussion.

The secretions of the various glands and the different body metabolites contain a wide range of chemical compounds such as amines, aldehydes, ketones, carbohydrates, alcohols, phenols, fatty acids and esters. The optimal method for detecting each of these classes will depend on their specific chemical properties, and no one method will be optimal for them all. The biologically active compounds may belong to any of these classes. Several studies (see reviews mentioned in the Introduction) have produced results on the chemical composition of secretions from various species, but very few of them have been able to pinpoint and classify the biologically active compounds.

In the present discussion it is important to remember that the method of analysis available to the author does not provide a complete chemical profile of lion urine. Therefore it is likely that many more compounds will be identified in lion urine as different methods of analysis are employed. Whether the biologically active compounds are present in the fraction identified and listed in this study is not known at the present time, but possible candidates can be identified.

The group-living lion has three potentially different situations in which chemical signals can be used. Firstly, a scent mark can be deposited in the presence of other members of the pride and be intended for communication with those other members i.e. as an instantaneous communication in which the signal life-time needs only be short. Secondly, as lion groups often split up into smaller units which move independently throughout the territory, a scent mark deposited by a lion in one of these units could be intended for members moving around in the other units thereby helping them to keep track of each other. This signal would have to last for at least a few hours up to a couple of days in order to be effective. Thirdly, lions may leave scent marks within and on the borders of their territories in order to inform potential intruders that the area is already occupied.

These marks have to last for a long time, weeks if not months, in order to be an effective means of communication. Supposing the lion is capable of producing only one type of scent mark, the mark would have to be composed of a range of chemical compounds, some with very high, some with medium and some with low volatility.

No published data on chemical composition of lion urine are available. Brahmachary and Dutta (1981) erroneously quote Albone *et al.* (1977) in stating that putrescine (1,4-butanediamine) and cadaverine (1,5-pentanediamine) are present in lion marking fluid, when in fact they wrote (Albone *et al.* 1977, p. 37): "In addition, ammonia, putrescine and cadaverine are major contributors to the *anal sac secretions* of both the lion and the red fox." (my italics). However this misunderstanding may be based on the fact that at that time, marking fluid was thought to contain secretions from the anal sacs.

Amines.

Special attention has been given to amines by other workers when they have analysed scent samples from the *Panthera* species (Brahmachary and Dutta 1979, 1981, 1987; Banks *et al.* 1992). Whether or not this special attention is justified in that amines play an important role in the communication system of these species still remains to be seen.

Trimethylamine (No 1) was detected in samples from three of the five male lions (Table 3.2) and in eight of the 13 female lions, as well as in the sample of bladder urine from a KBH female (KB2). 3-methyl-1-butylamine (No 16) was found in two male lions (Napoleon and KolmHan). Compound 72 (azetidine ?) was found once in a sample of bladder urine (KB2 - Table 3.3). From this evidence it appears that at least one amine (Trimethylamine) is regularly though not universally present in lion urine. Trimethylamine is a highly volatile compound and it could therefore be used for short term communication purposes, but whether or not it plays a role in the scent communication system of lions is impossible to say at this stage.

Non-amines.

No studies are available on the non-amine fraction of lion urine. The most common of the non-amines found in lion urine in this study were acetone (No 4), 2-butanone (No 10), 1-pentene (No 26), heptanal (No 31), 2-pentylfuran (No 37), 1,2-cyclooctadiene (No 51) and diethylbenzene (No 62). All of these compounds were detected in most samples from most of the individuals. A whole range of other compounds were present but much less

frequently than those mentioned above. Some of these compounds will be excretions from the normal metabolic process, whereas others will have a different origin. The role, if any, of these individual compounds are still unknown, but a few guesses can be ventured on the basis of the results presented in the previous section.

Can lions use scent marks deposited by foreign animals to identify their individual identity, sex or species and if so how do they do it?

Out of the 58 (81) chemical compounds identified in this study from the lion samples, seven were almost universally present and it is therefore possible that some or all of these compounds could carry a message of species identity individually or in combination. The seven compounds are listed in the paragraph above. Great care has to be taken, however, when such conclusions are drawn. An example of this was shown by Brahmachary and Dutta who in 1979, after having identified phenylethylamine (PEA) repeatedly in tiger urine samples, stated that "PEA is likely to be a biochemical marker at the species level" (Brahmachary and Dutta 1979). The same authors later found PEA to be present in leopard urine as well (Brahmachary and Dutta 1984), and since these two species occurs sympatrically it is impossible for PEA alone to act as a marker on the species level.

Of the compounds identified in only male lion samples, none was present in all the males, and it is therefore uncertain whether one or more of these compounds carries a message of "Maleness". Only if other lions could "remember" this list of compounds and by the presence of any one of them identify a given scent mark as originating from a male lion could this system work. It is also conceivable that there exists a universal male identifying compound in the urine which has not been discovered by the method of analysis available to the author in this study, and that this universal compound could be a derivative of the male hormone testosterone. Likewise the compounds found in only female lion samples were far from common to all the female samples analyzed, so again they could only carry a message of "Femaleness" if the presence of any one of these compounds was sufficient to identify a scent mark as having been deposited by a female lion.

Though no specific "male" compound was identified it was found that males overlapped other males significantly more in compound composition than they did with

females. However, a similar significant difference was not found for the females, so it is still unclear what role, if any, the overall compound composition plays in the identification of sex.

On the quantitative level a difference was detected between the two sexes in that males had a significantly higher absolute concentration of 2-butanone (No 10) than females. Females had a significantly higher percentage concentration of acetone (No 4) than males. Further minor non-significant differences in the percentage concentration between the sexes were present. If lions are able to detect such differences a high absolute level of 2-butanone coupled with a low relative level of acetone could signal "Maleness" and the opposite relationship could signal "Femaleness". Or any of these two factors alone could contribute to the sex identity of lion scent marks. Thus in combination qualitative and quantitative aspects of the scent marks are likely to be able to carry a message of sex identity.

Do the scent marks carry a message of identity for the individual lion? Some evidence for this was found in that no two individuals had an identical compound composition. It is therefore possible that the compound composition of a scent mark alone could hold the key to the identity of the animal. This was tentatively supported by the finding that 13 out of the 16 individual lions studied had a higher overlap within their own samples than their total composition overlapped those of the other individuals. However, only seven out of the 13 overlaps were significantly in favour of the "within overlap", so the conclusion is by no means clear cut.

All in all the results presented in the above section are in support of Hypothesis 5 which states that differences in chemical composition are present between scent marks and that these differences form the basis for the animal's ability to distinguish between marks.

3.5 Summary.

Scent communication in lions is a relatively little studied area of lion biology. In the present study several findings were reported which provide new insight into this area. It was found that apart from normal urination the two most common types of scent marking in lions are *Spraymarking* and *Scrape/urination*. Males had a higher

percentage of *Spraymarking* in their marking repertoire than females, whereas the two sexes had equal percentages of *Scrape/urination*. Males had higher rates of *Spraymarking* and significantly higher rates of *Scrape/urination* than females.

For the GIV males *Spraymarking* was seen predominantly in a territorial context and *Scrape/urination* showed no bias between territorial or social context. For the females both marking types were seen predominantly in the territorial context.

The lions were able to discriminate between two scent marks of different origin. No overall significant difference in either *Scenting* or *Flehmen* frequencies towards the experimental mark types were found between the sex/age groups. Generally all sex/age groups showed high interest in tiger and leopard marks.

58 chemical compounds were found in urine from the lions, and a number of these compounds was specific to either male or female urine. The chemical composition of male lion samples overlapped significantly more with other male lions than with female lion samples. Females did not overlap significantly more within their own sex group than with males. The majority of the lions overlapped more within their own samples than they did with samples from other lions, and for seven out of 16 lions this difference was significant.

Male lions had a significantly higher absolute concentration of 2-butanone than females, and females had a significantly higher relative concentration of acetone than did males.

Figure 3.1: Activity of KBH and GIV lions.
M: male, F: female.

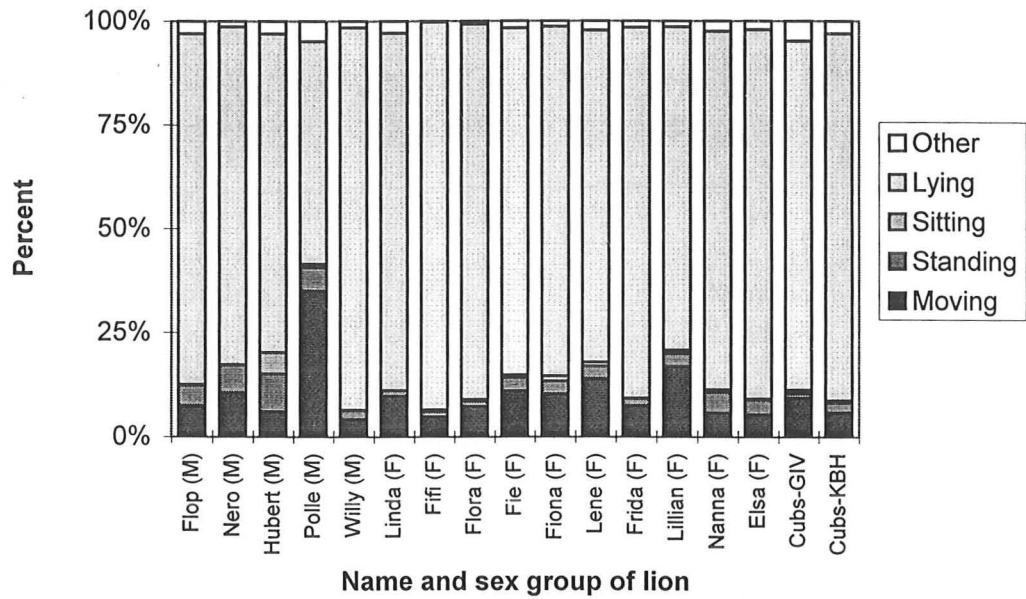
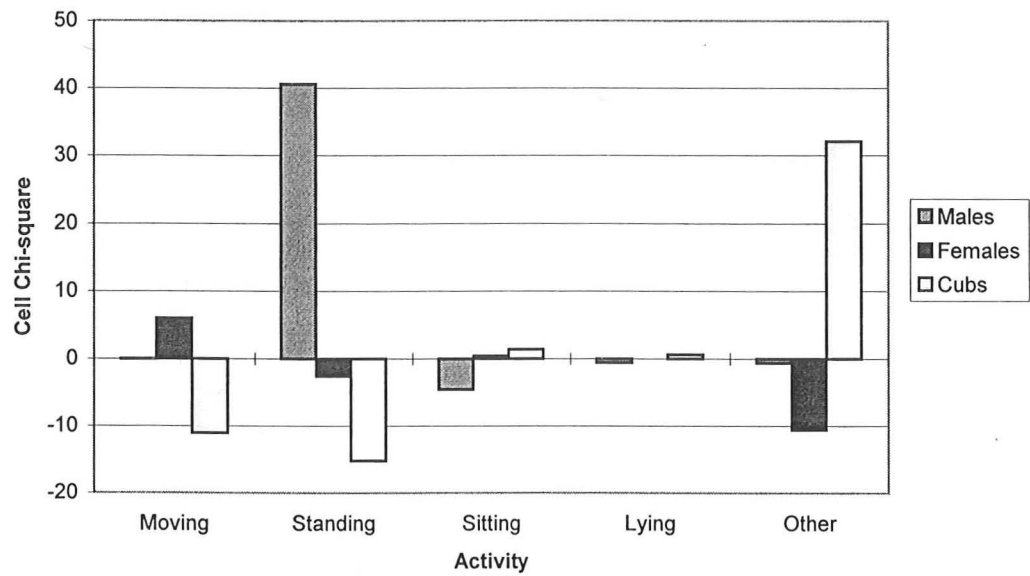


Figure 3.2: Activity differences between lion sex/age groups. Cell Chi-square values including direction of deviation.



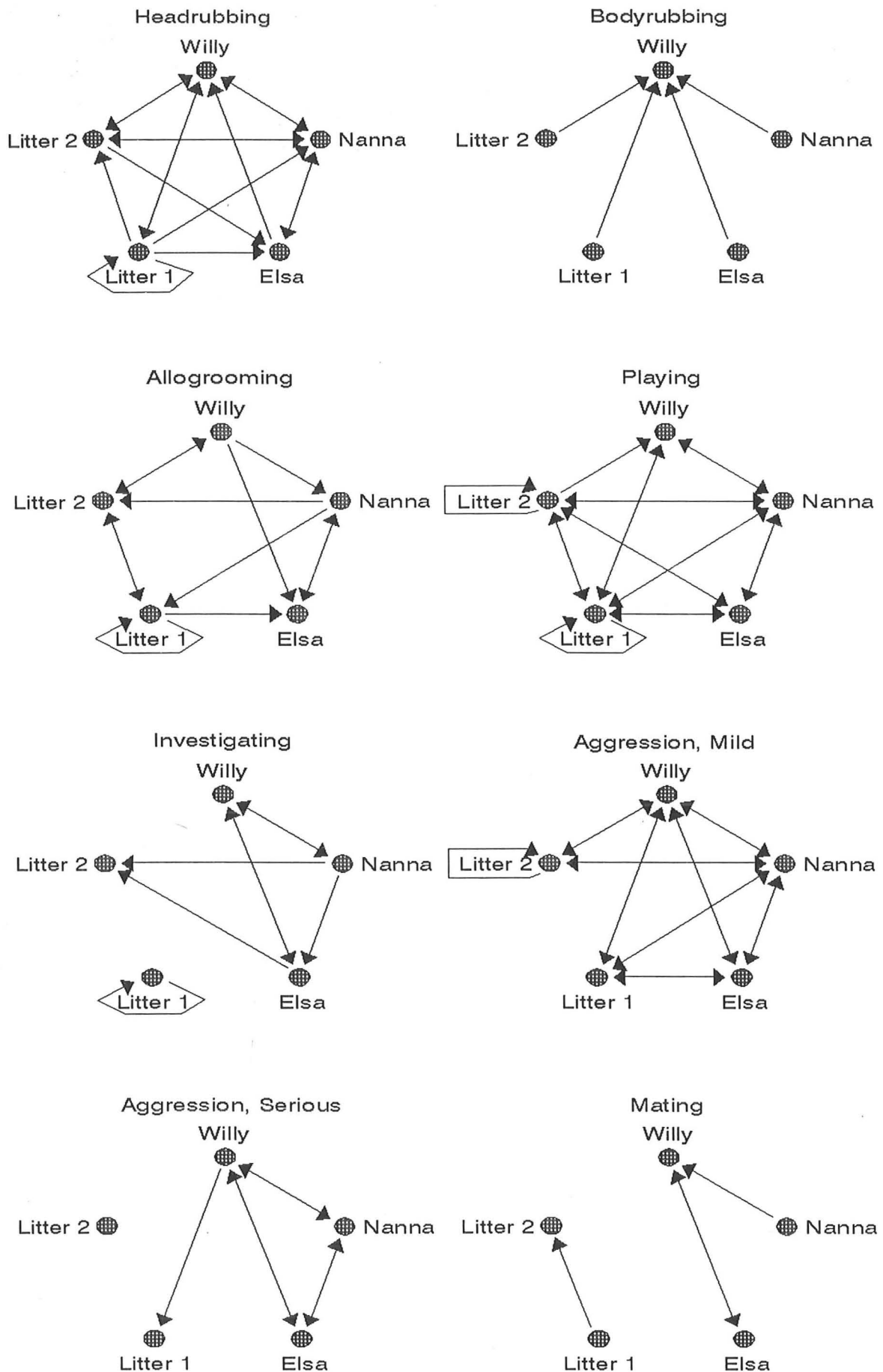


Figure 3.3: Qualitative social interactions between all individuals in the KBH lion pride. "Mating" includes Mock matings. Arrows indicate direction of interactions from the Actor to the Target. M: Willy, F: Nanna and Elsa.

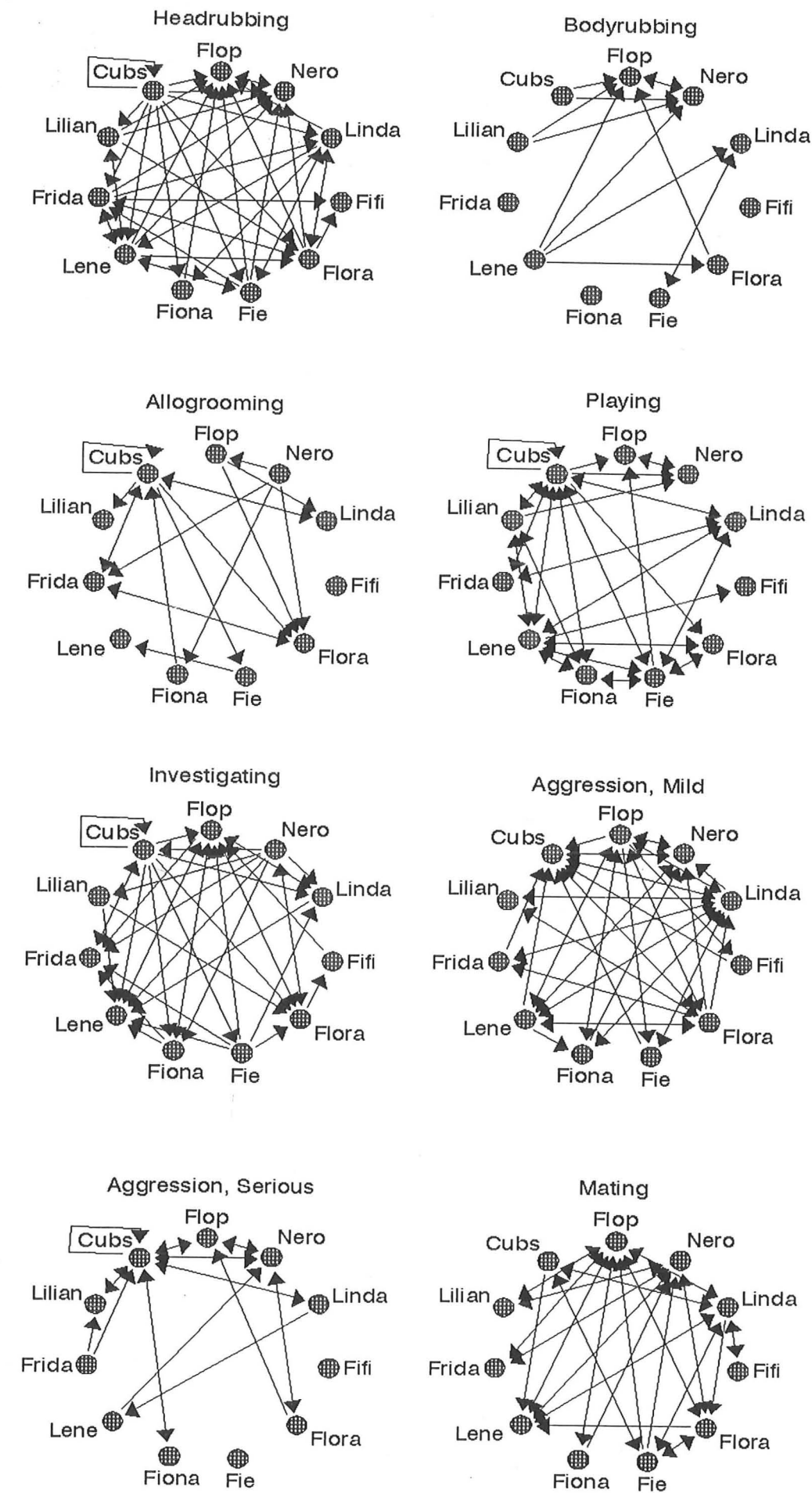


Figure 3.4: Qualitative social interactions between all individuals in the GIV lion pride. "Mating" includes Mock matings. Arrows indicate direction of interactions from the Actor to the Target. M: Flop and Nero, F: Linda, Fifi, Flora, Fie, Fiona, Lene, Frida, Lillian, Nanna and Elsa.

Figure 3.5: Percentage distribution of the six most frequent social behaviours performed by the KBH and GIV lions.

M: male, F: female.

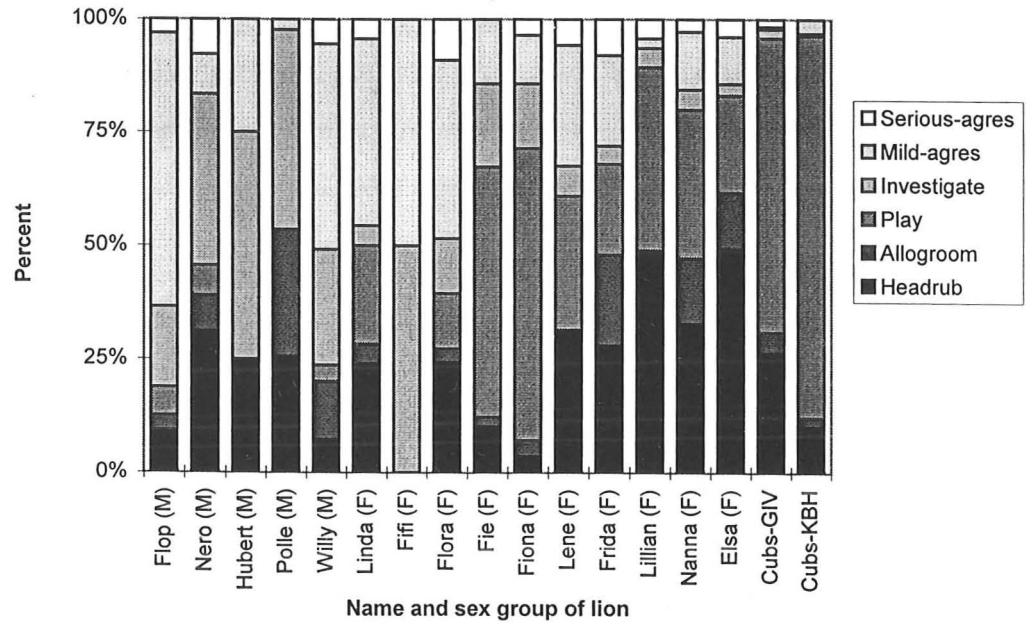


Figure 3.6: Social behaviour differences of lion sex/age groups.
Cell Chi-square values including direction of deviation.

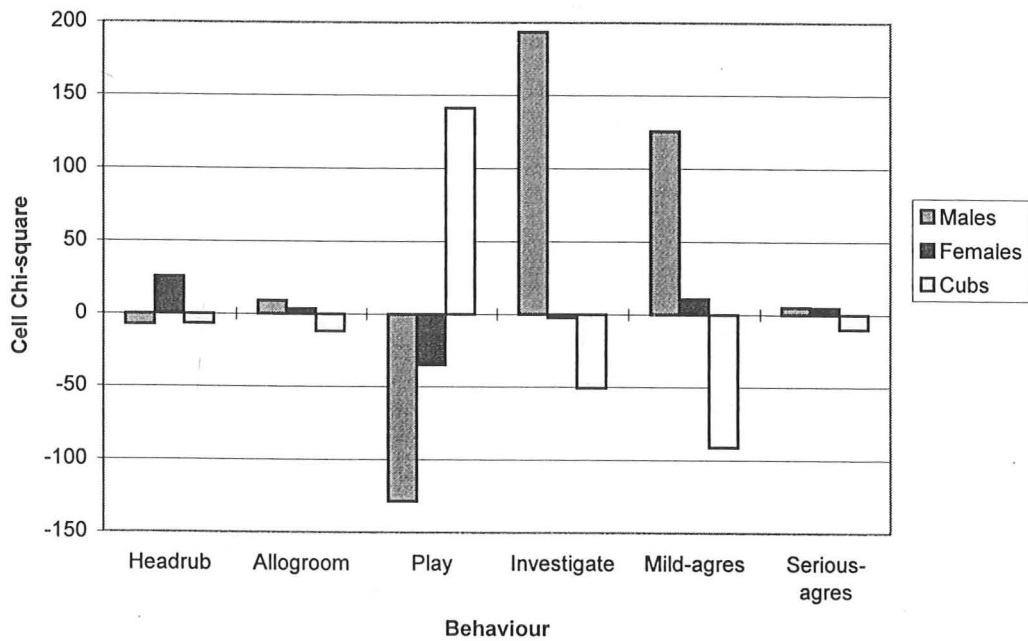


Figure 3.7: Number of Non-Aggressive and Aggressive social interactions between sex/age groups in KBH lions. Actor vs. Target groups are indicated below the x-axis.

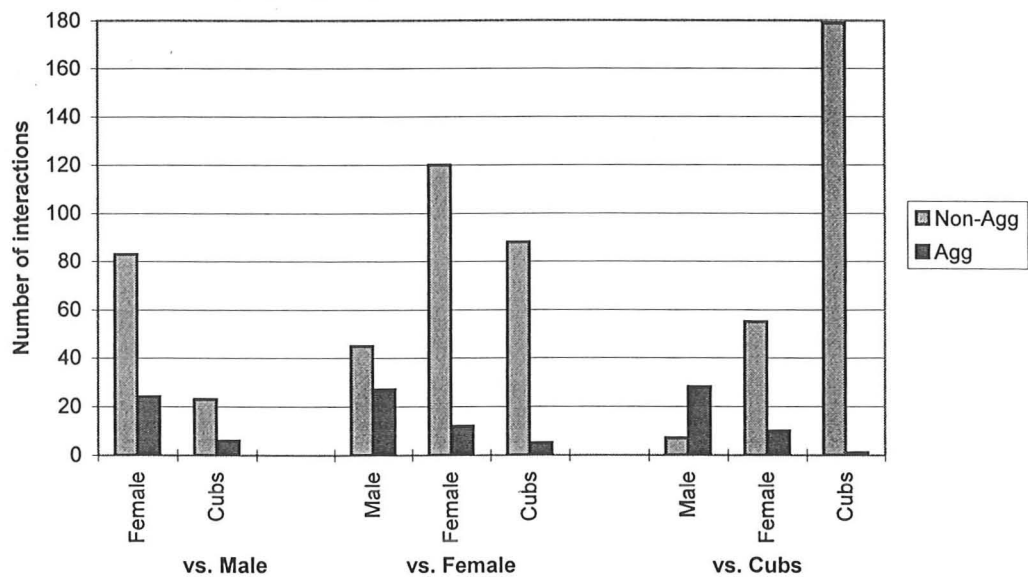


Figure 3.8: Number of Non-Aggressive and Aggressive social interactions between sex/age groups in GIV lions. Actor vs. Target individuals or groups are indicated below the x-axis. The two adult males Flop and Nero are shown individually.

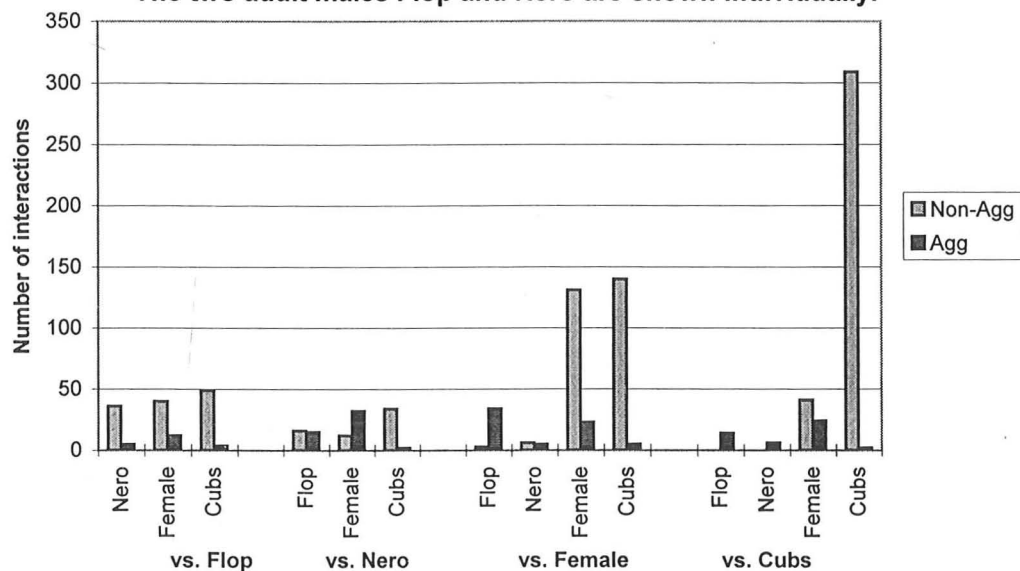


Figure 3.9: Percentage distribution of the different mark types for KBH and GIV male lions.

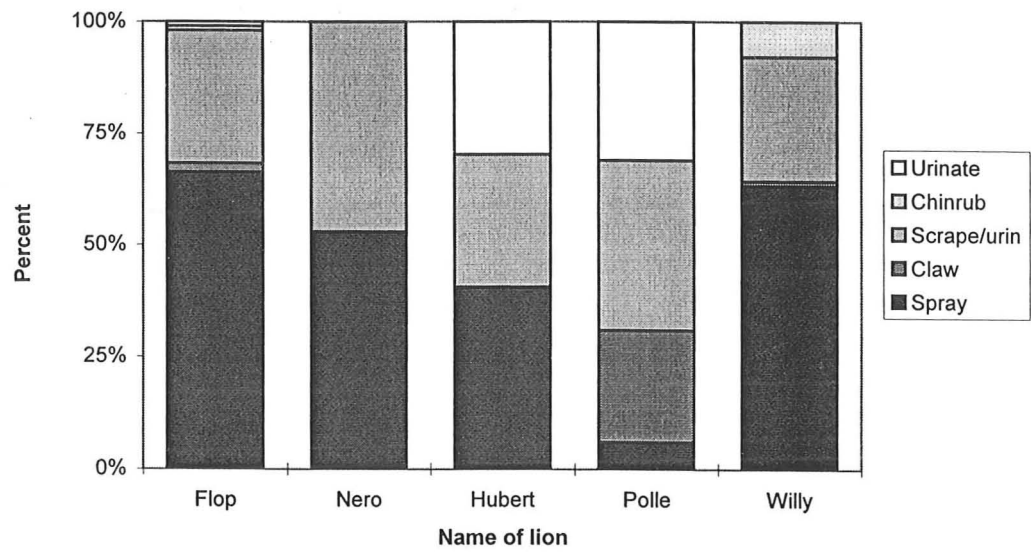


Figure 3.10: Percentage distribution of the different mark types for KBH and GIV female lions.

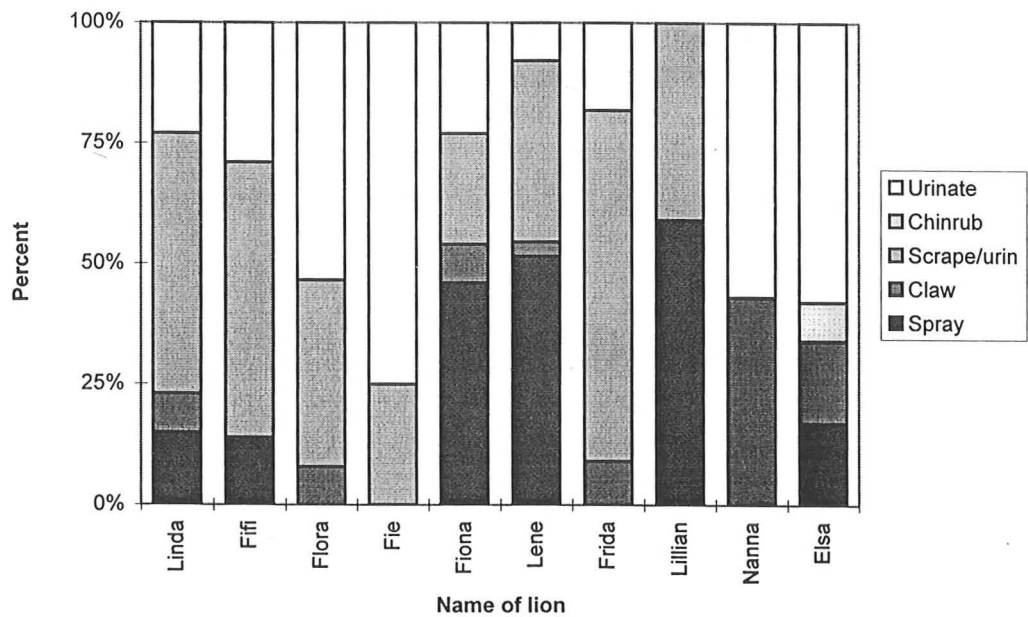


Figure 3.11: Percentage of Spraymarking for male and female lions in KBH and GIV. Black lines show mean value for each sex group.

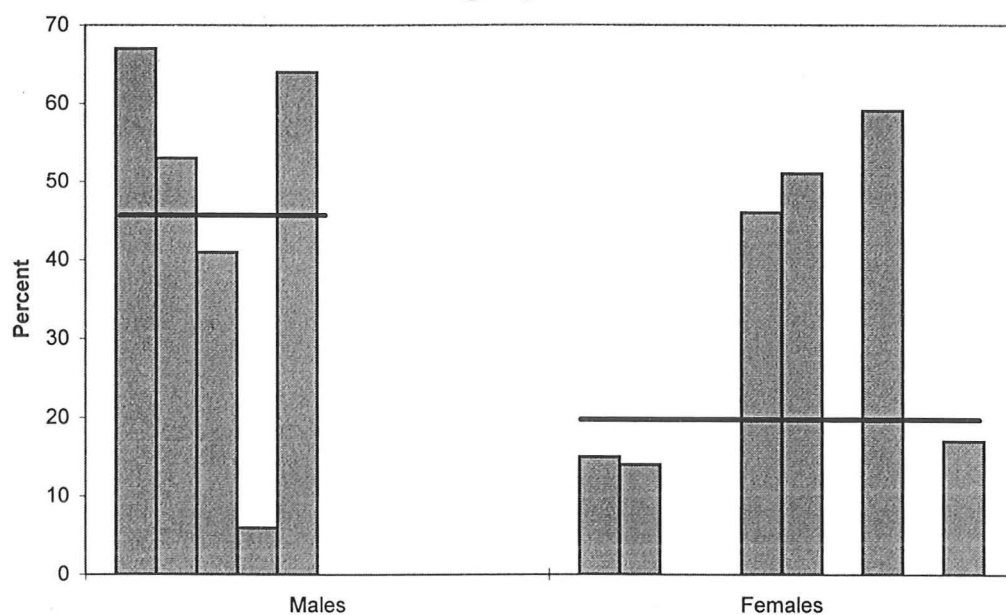


Figure 3.12: Percentage of Scrape/urination for male and female lions in KBH and GIV. Black lines show mean value for each sex group.

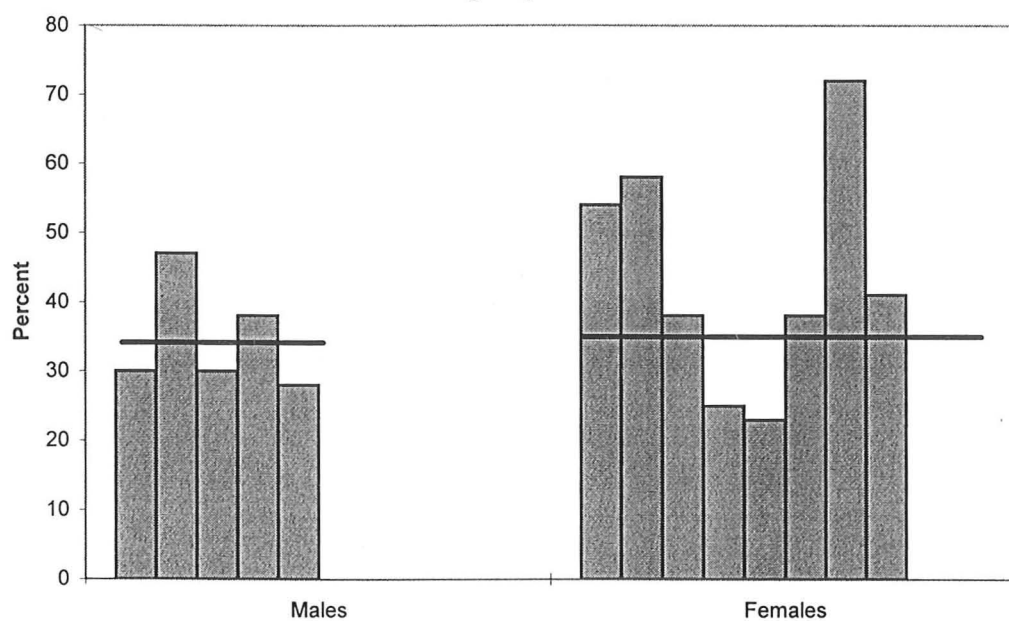


Figure 3.13: Marking rates of the different mark types for KBH and GIV male lions.

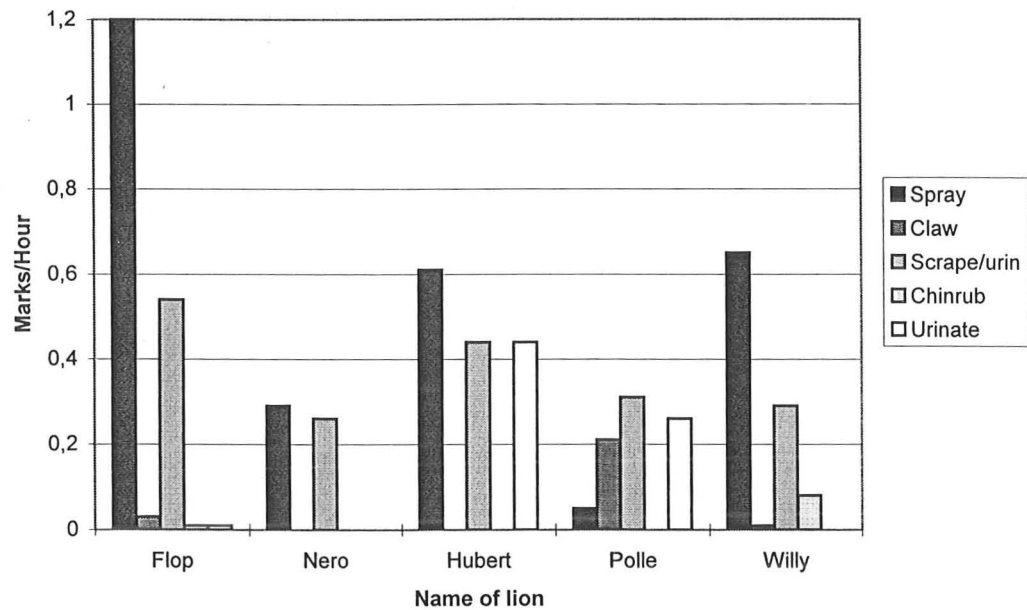


Figure 3.14: Marking rates of the different mark types for KBH and GIV female lions.

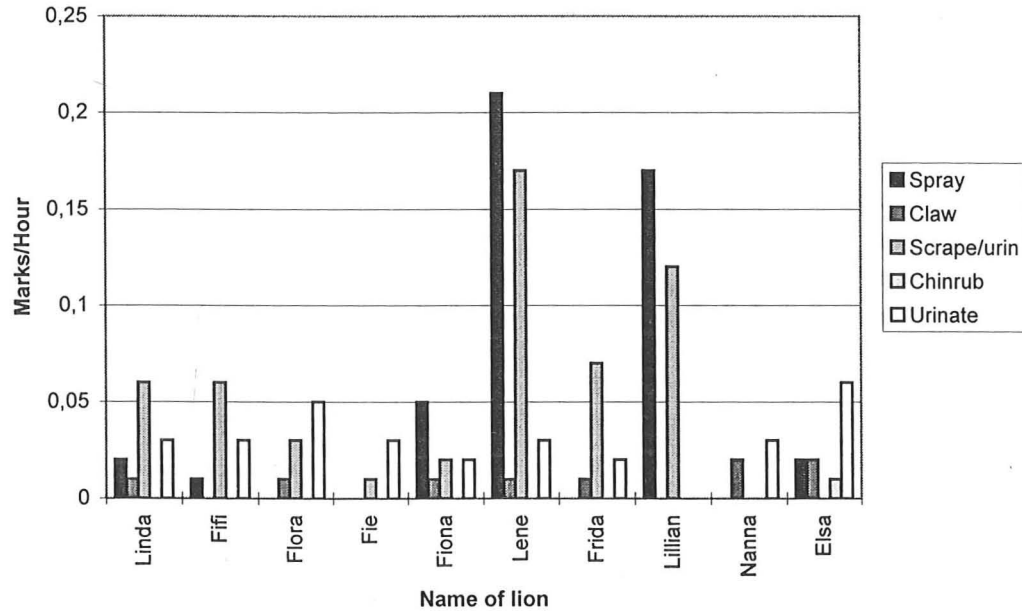


Figure 3.15: Rates of Spraymarking for male and female lions in KBH and GIV. Black lines show mean value for each sex group.

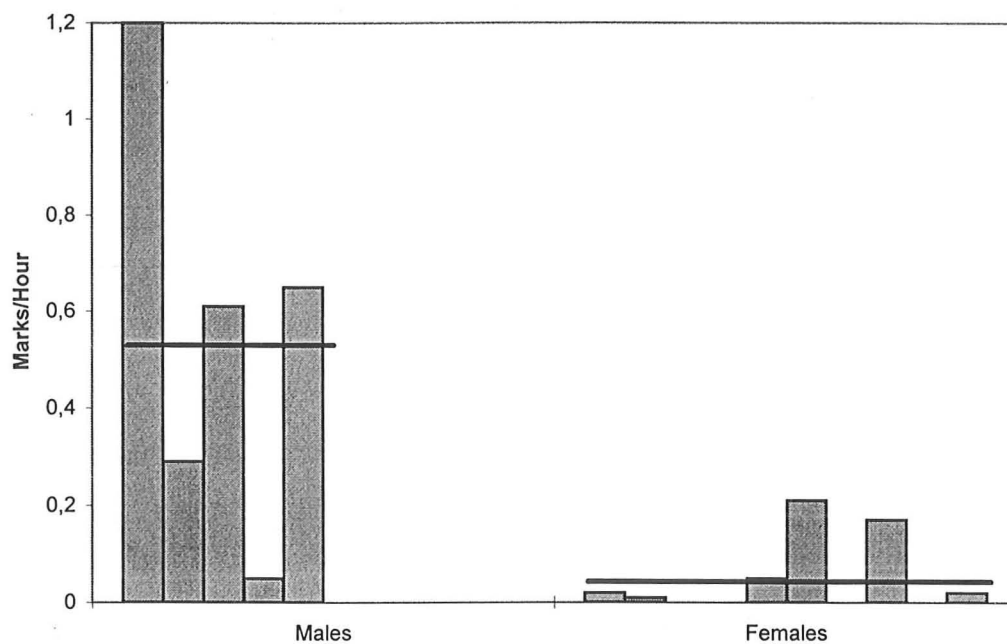


Figure 3.16: Rates of Scrape/urination for male and female lions in KBH and GIV. Black lines show mean value for each sex group.

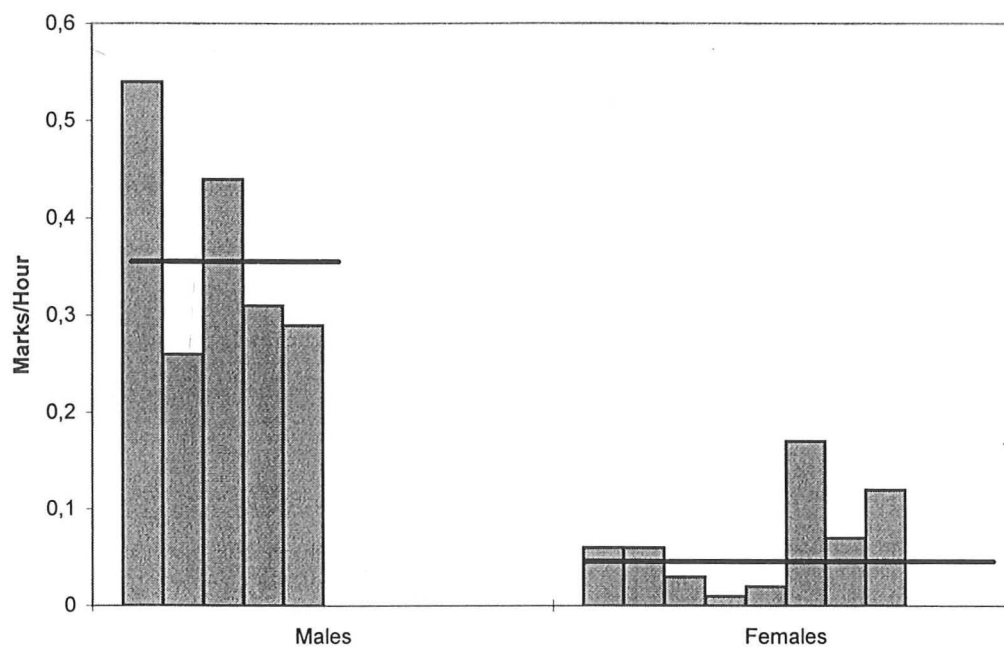


Figure 3.17: Context of Spray and Scrape/urination marks for GIV male and female lions.

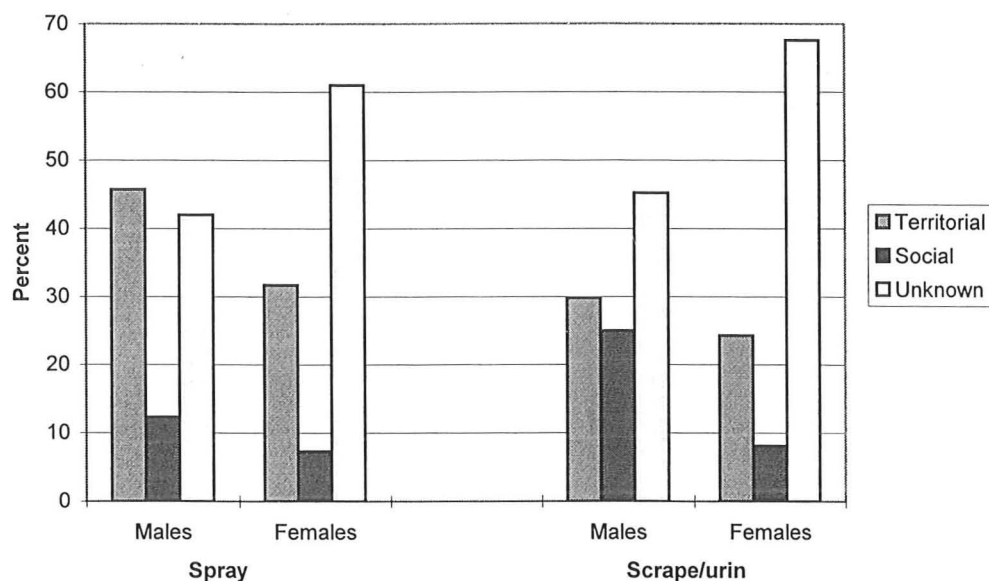


Table 3.1: Percentage of Spray and Scrape/urination marks observed in either territorial or social contexts for GIV male and female lions. (G-test).

	Spray		Scrape/urination		
	Territorial	Social	Territorial	Social	
Males	78.7	21.3	54.3	45.7	***
Females	81.3	18.8	75.0	25.0	NS
	NS		**		

Figure 3.18: Combined data for KBH and GIV, Indoor and Outdoor scent presentation experiments for lions. Cell Chi-square values including direction of deviation.

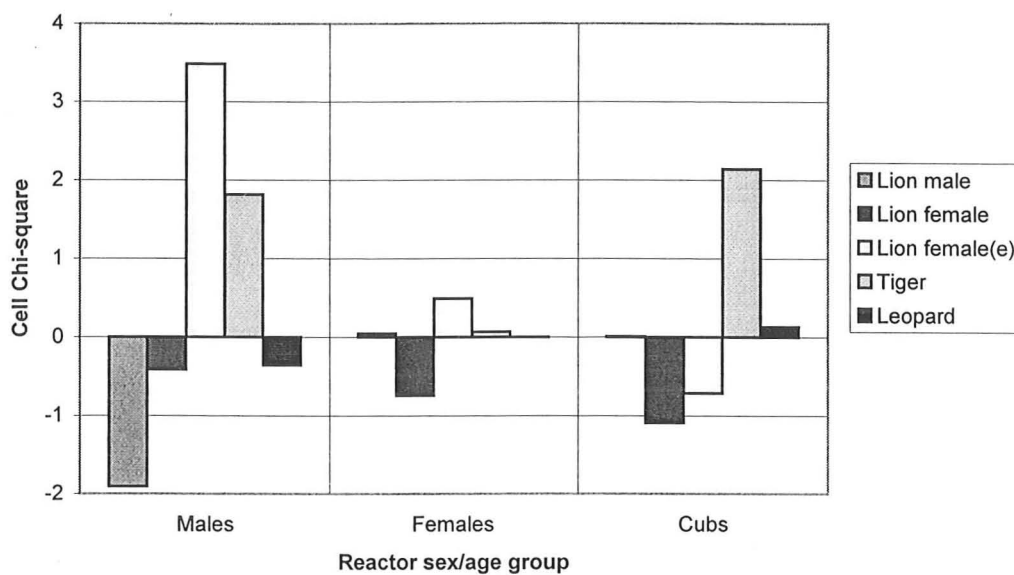


Figure 3.19: Indoor scent presentations for KBH lions. Cell Chi-square values including direction of deviation.

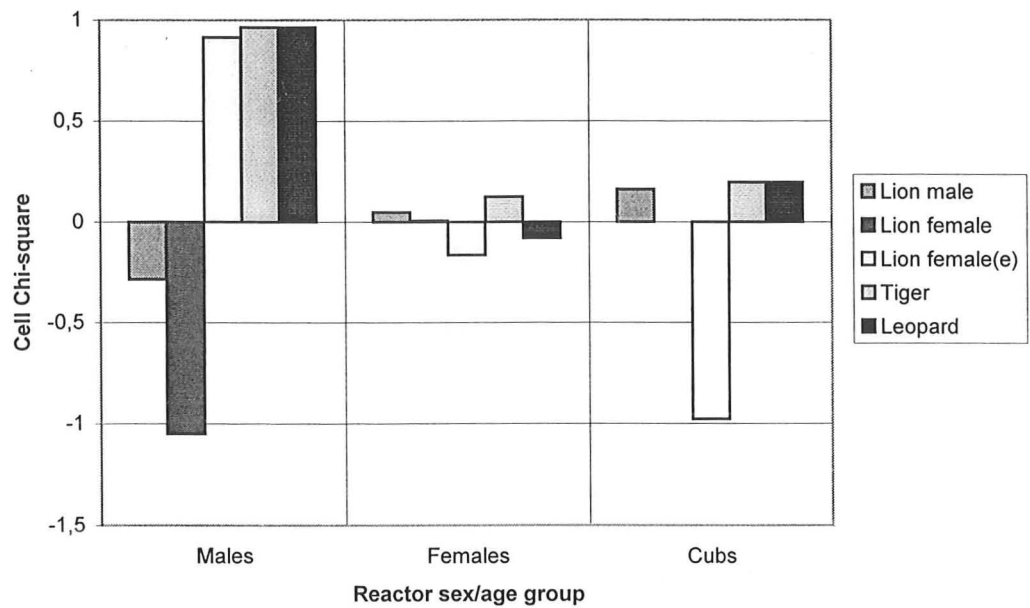


Figure 3.20: Outdoor scent presentations for KBH lions. Cell Chi-square values including direction of deviation.

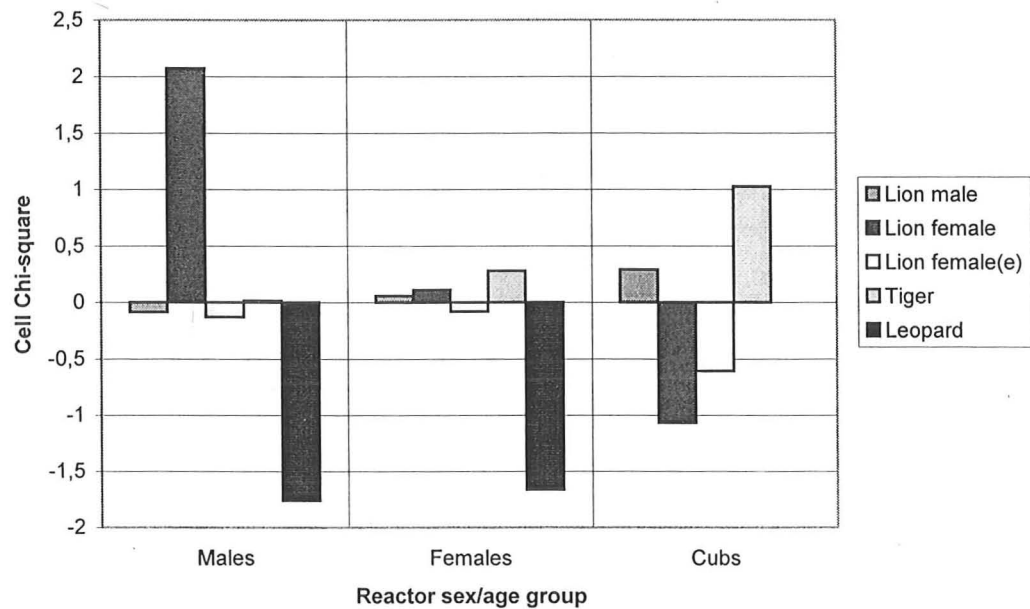


Figure 3.21: Indoor scent presentations for GIV lions. Cell Chi-square values including direction of deviation.

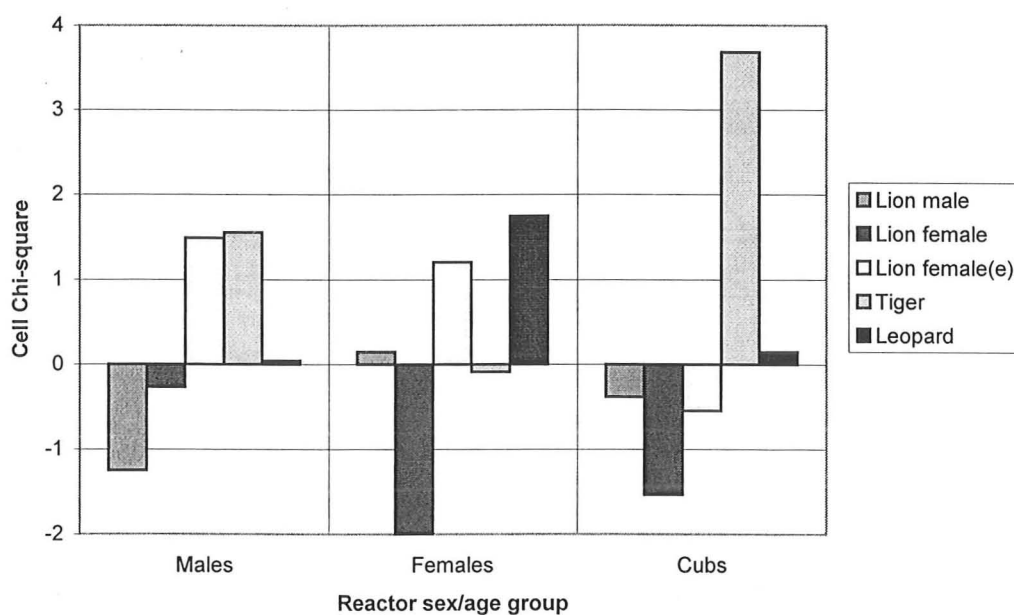


Figure 3.22: Outdoor scent presentations for GIV lions. Cell Chi-square values including direction of deviation.

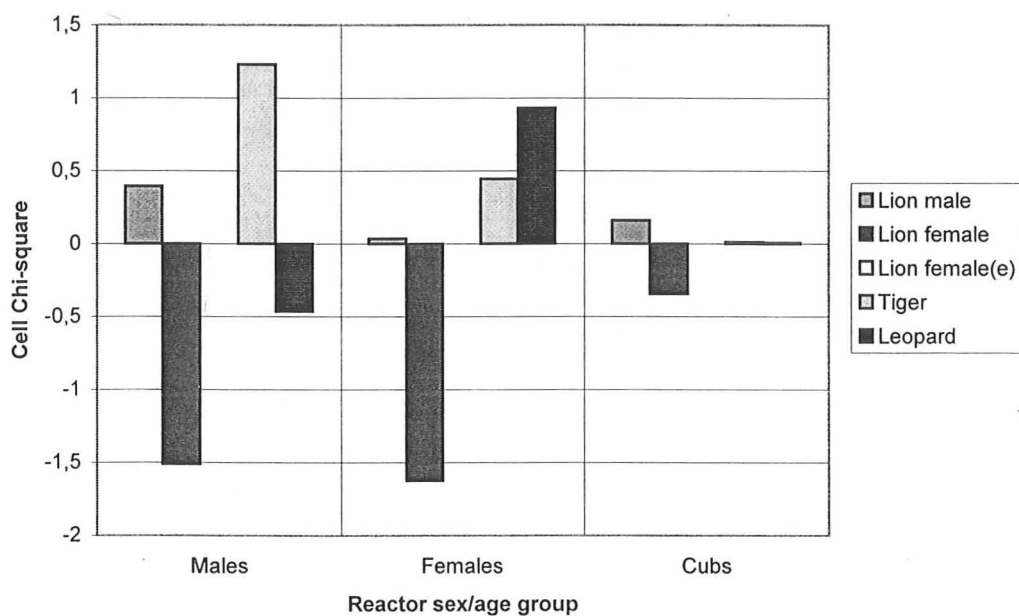


Figure 3.23a: Frequency of Scentings (per 4 hrs presentations) for male lions in KBH and GIV when investigating different experimental mark categories.

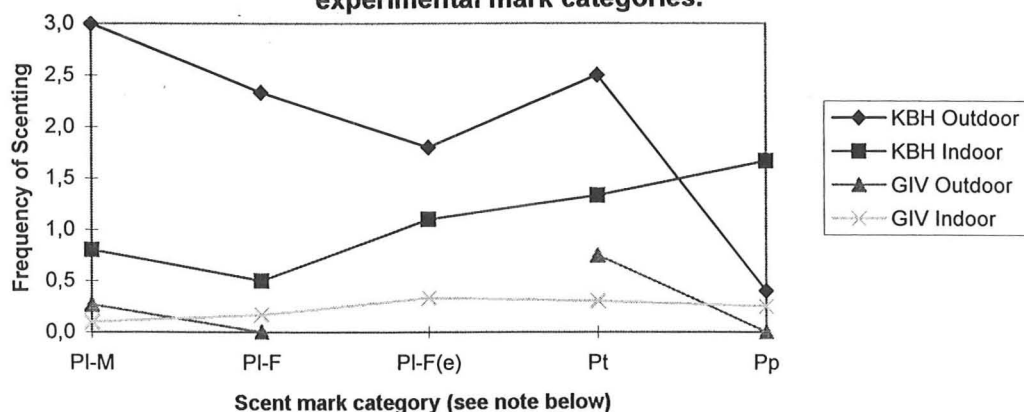


Figure 3.23b: Frequency of Scentings (per 4 hrs presentations) for female lions in KBH and GIV when investigating different experimental mark categories.

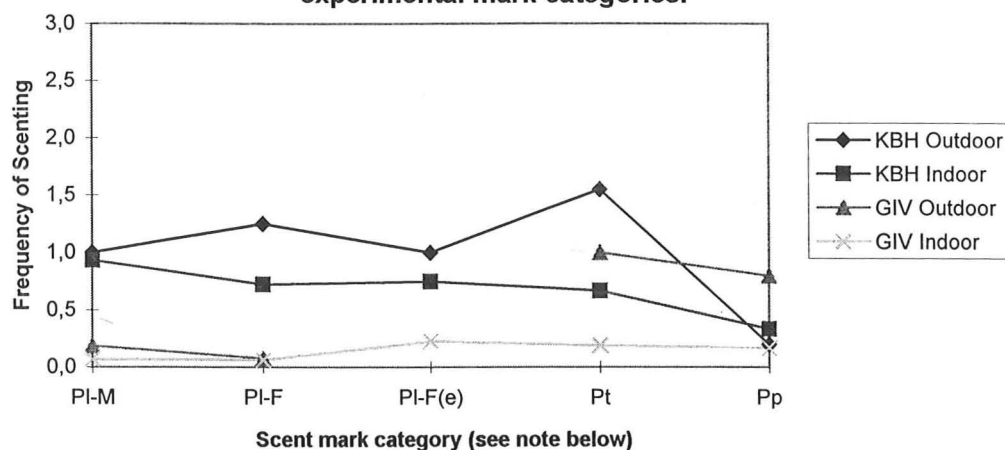
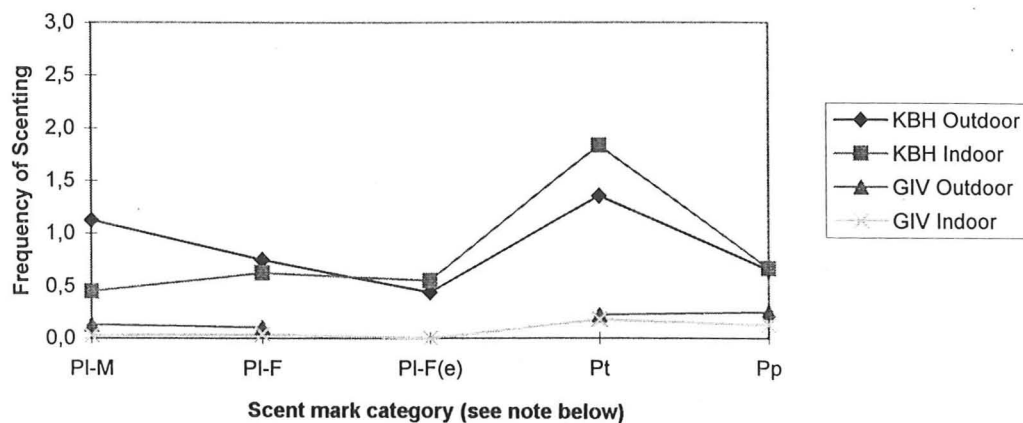


Figure 3.23c: Frequency of Scentings (per 4 hrs presentations) for lion cubs in KBH and GIV when investigating different experimental mark categories.



Note to Figures 3.23a,b,c:

Scent mark categories: PI-M: Lion male marks, PI-F: Lion female marks, PI-F(e): Lion female estrous marks, Pt: Tiger marks, Pp: Leopard marks.

Figure 3.24a: Frequency of Scentings (per 4 hrs presentations) for lion sex groups when investigating different experimental mark categories in KBH Outdoor experiments.

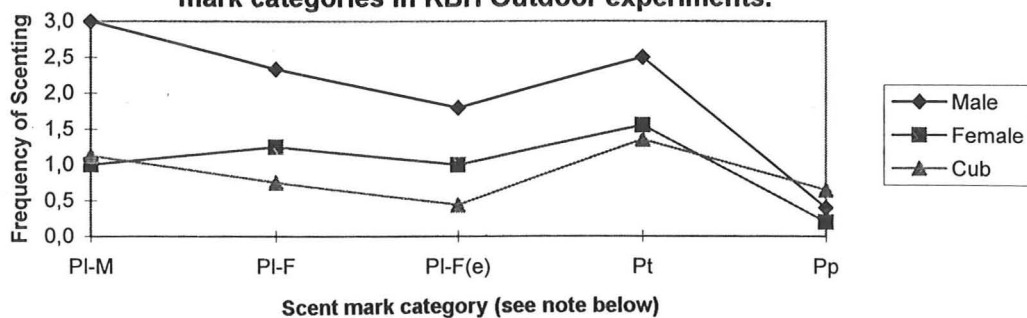


Figure 3.24b: Frequency of Scentings (per 4 hrs presentations) for lion sex groups when investigating different experimental mark categories in KBH Indoor experiments.

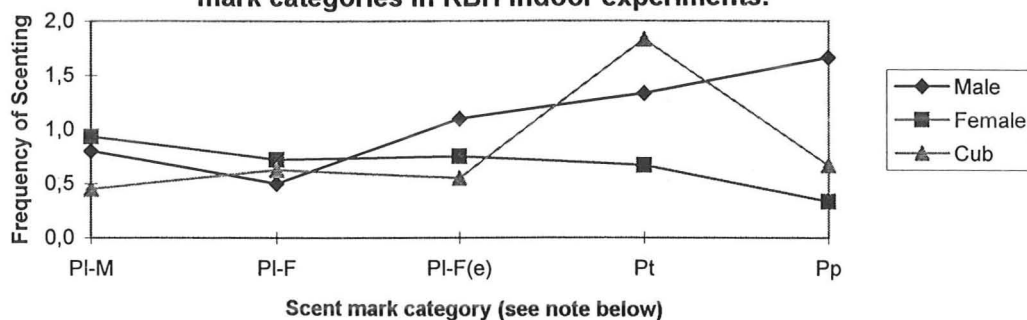


Figure 3.24c: Frequency of Scentings (per 4 hrs presentations) for lion sex groups when investigating different experimental mark categories in GIV Outdoor experiments.

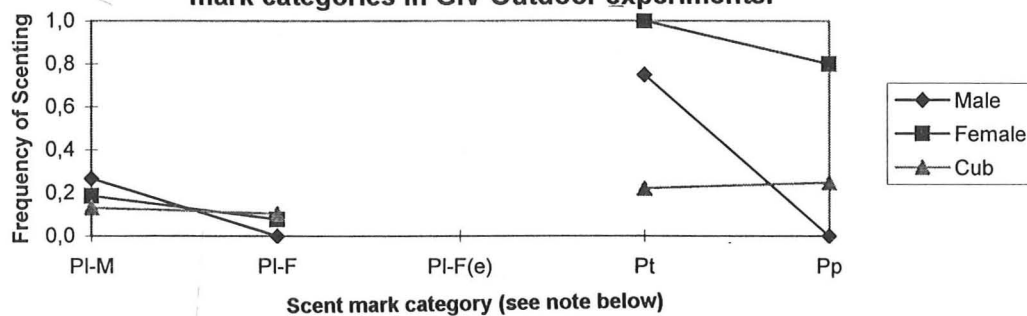
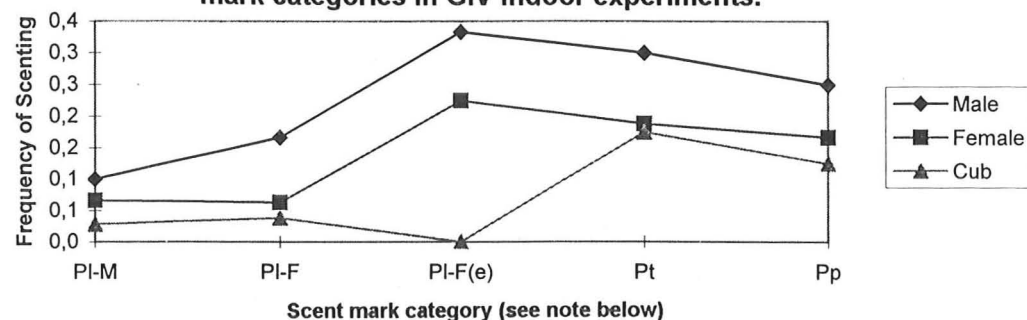


Figure 3.24d: Frequency of Scentings (per 4 hrs presentations) for lion sex groups when investigating different experimental mark categories in GIV Indoor experiments.



Note to Figures 3.24a,b,c,d:

Scent mark categories: PI-M: Lion male marks, PI-F: Lion female marks, PI-F(e): Lion female estrous marks, Pt: Tiger marks, Pp: Leopard marks.

Figure 3.25a: Frequency of Flehmens (per 4 hrs presentations) for male lions in KBH and GIV when investigating different experimental mark categories.

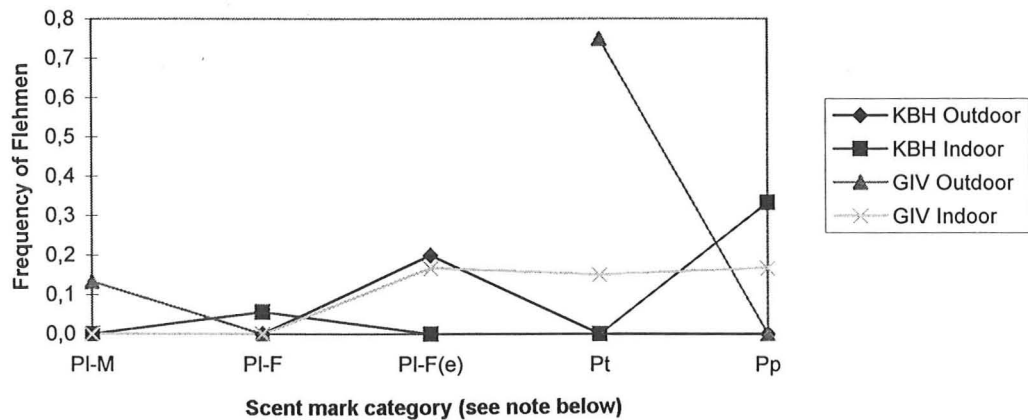


Figure 3.25b: Frequency of Flehmens (per 4 hrs presentations) for female lions in KBH and GIV when investigating different experimental mark categories.

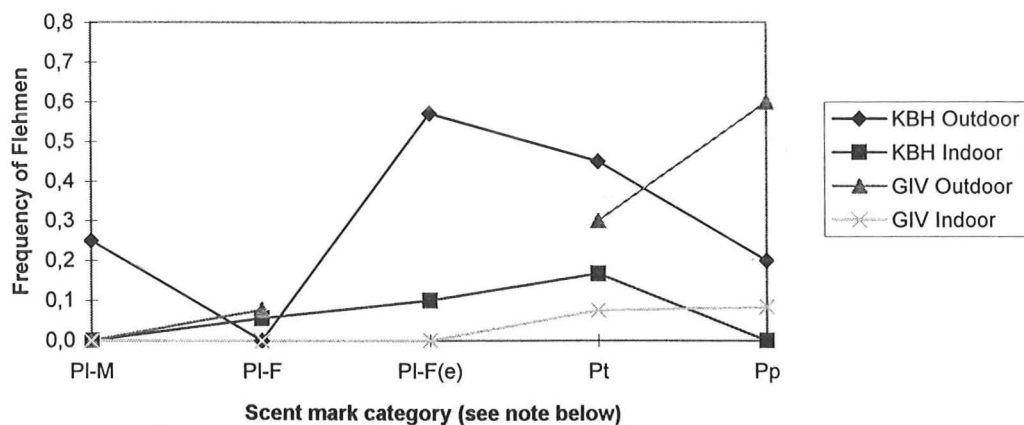
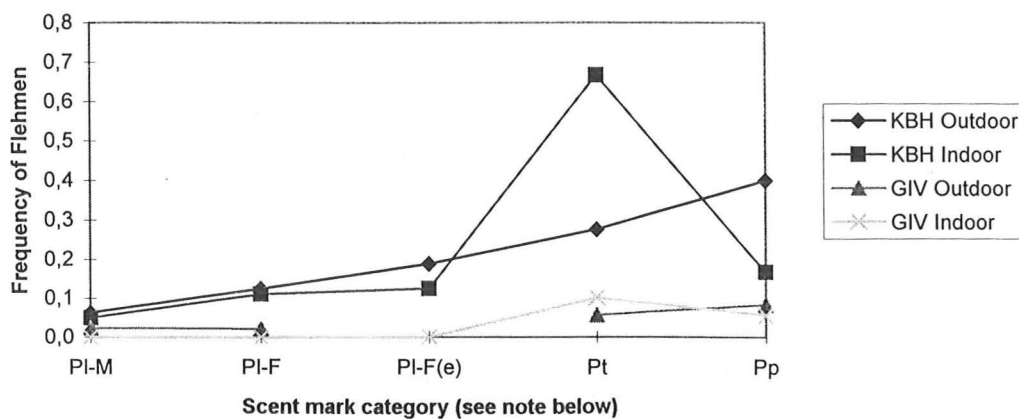


Figure 3.25c: Frequency of Flehmens (per 4 hrs presentations) for cub lions in KBH and GIV when investigating different experimental mark categories.



Note to Figures 3.25a,b,c:

Scent mark categories: PI-M: Lion male marks, PI-F: Lion female marks, PI-F(e): Lion female estrous marks, Pt: Tiger marks, Pp: Leopard marks.

Figure 3.26a: Frequency of Flehmens (per 4 hrs presentations) for lion sex groups when investigating different experimental mark categories in KBH Outdoor experiments.

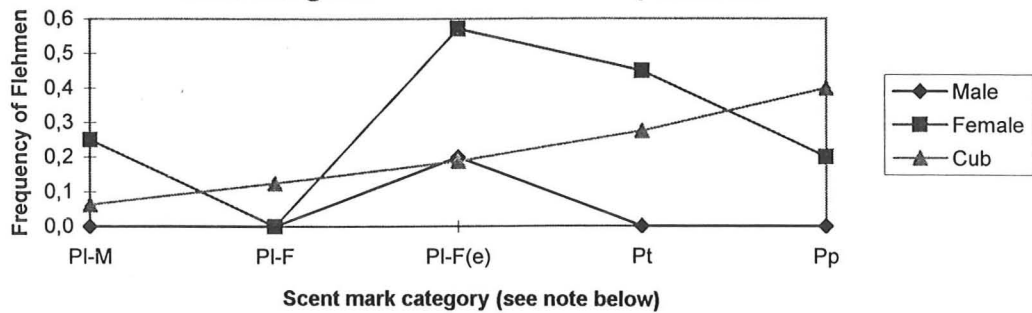


Figure 3.26b: Frequency of Flehmens (per 4 hrs presentations) for lion sex groups when investigating different experimental mark categories in KBH Indoor experiments.

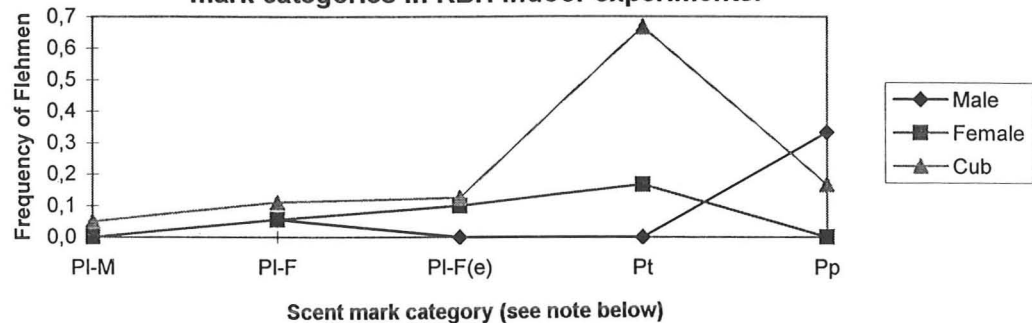


Figure 3.26c: Frequency of Flehmens (per 4 hrs presentations) for lion sex groups when investigating different experimental mark categories in GIV Outdoor experiments.

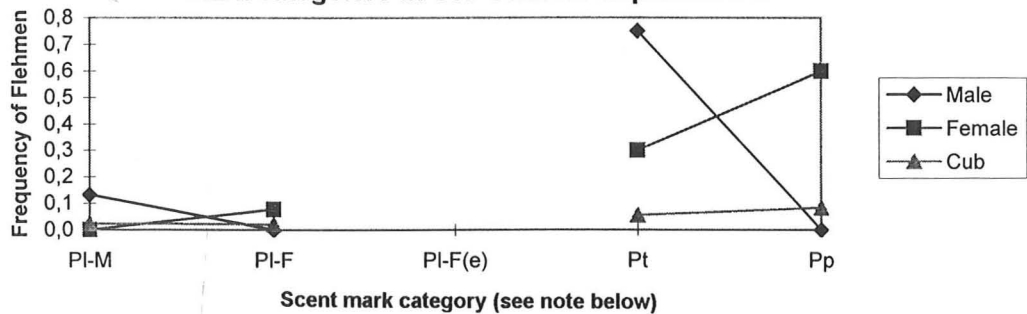
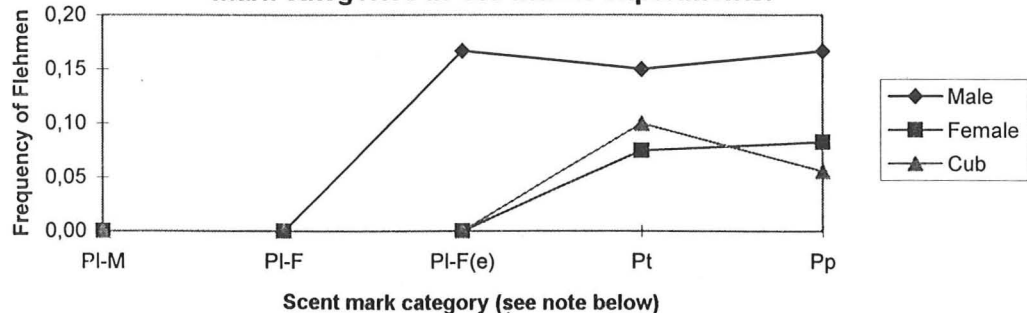


Figure 3.26d: Frequency of Flehmens (per 4 hrs presentations) for lion sex groups when investigating different experimental mark categories in GIV Indoor experiments.



Note to Figures 3.26a,b,c,d:

Scent mark categories: PI-M: Lion male marks, PI-F: Lion female marks, PI-F(e): Lion female estrous marks, Pt: Tiger marks, Pp: Leopard marks.

Figure 3.27: Investigation of indoor experimental marks by KBH lions. Like 1, 2 and 3 are identical marks, Odd is of different origin. Like 1 is the first mark encountered of its kind on a day, Like 2 the second and Like 3 the third.

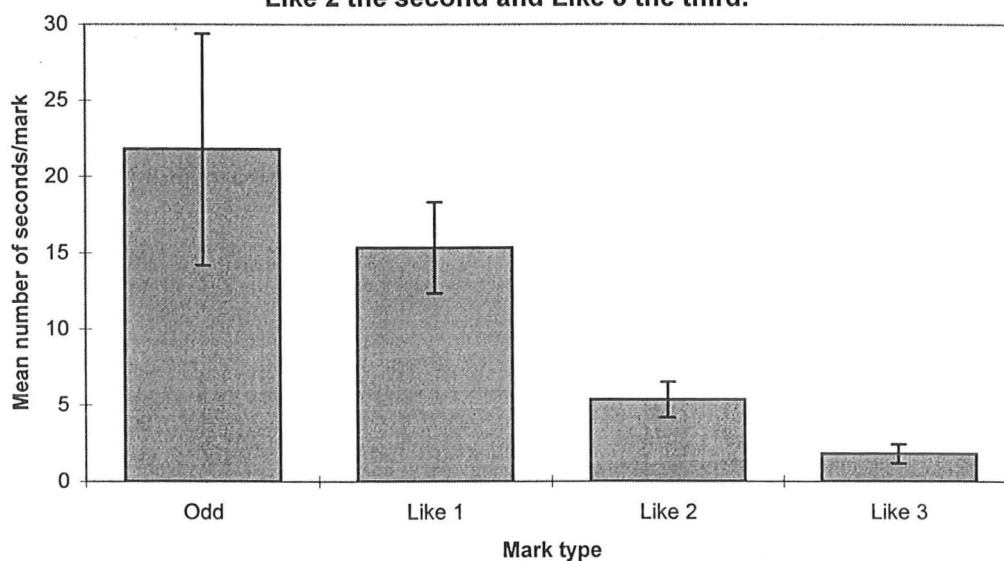


Figure 3.28: Investigation of indoor experimental marks by GIV lions. Like 1, 2 and 3 are identical marks, Odd is of different origin. Like 1 is the first mark encountered of its kind on a day, Like 2 the second and Like 3 the third.

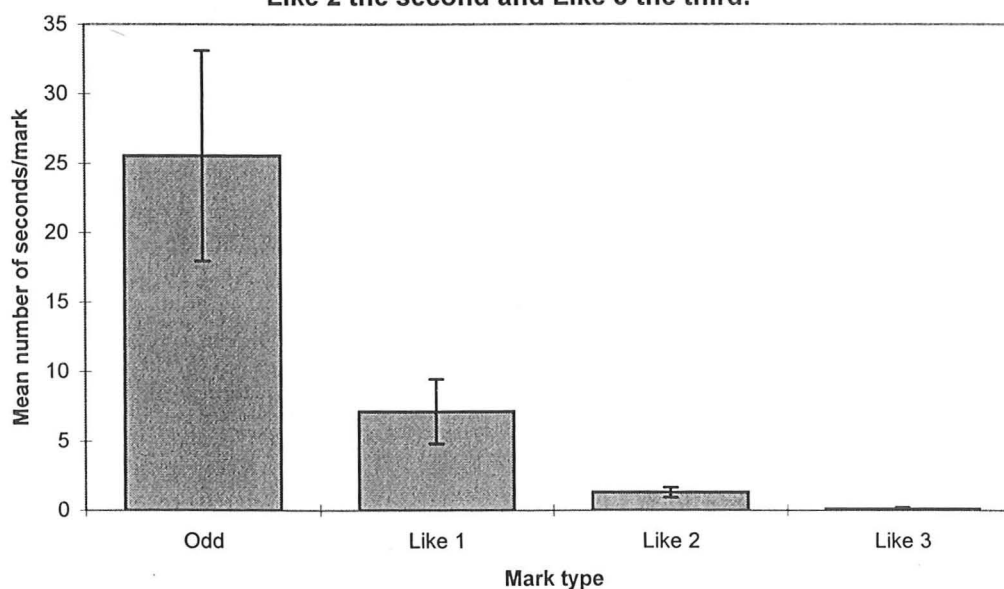


Table 3.2: Compounds present in the urine of male (n=5) and female (n=13) lions. X denote the presence of the compound in one or more of the samples analyzed. "0" indicates that the compound was present in both the sample and in a corresponding control sample (laboratory air), and a "S" indicate that the compound was present in both the sample and in the "water-sawdust" extraction. At the bottom of the table the number of samples analyzed for each individual is shown as is the total number of compounds found (excluding those also found in laboratory air controls and "water-sawdust" extraction samples). "Retention time" is defined as the time taken for each analyte to emerge from the chromatographic column (here given in minutes).

			Lion males					Lion females												
No.	Retention time	Name	Pol	Ner	Flo	Nap	KoIM	Lin	Fif	Len	Fie	Gur	Nan	Fio	Nat	Npo	Nel	Net	Lil	Nin
1	1.126	trimethylamine			X	X	X			X			X		X	X	X	X	X	X
2	1.376	pentane	0	0	0	0		0	X	X	0	0	0	0	0	0				
3	1.426	ethanol							X	X										
4	1.642	acetone	0	0	X	0	X	0	0	0	0	0	0	0	X	0	X	X	X	X
5	1.742	carbon disulfide	0	0	0	0		0	0	0	0	0	0	0	0	0				
6	1.909	methylene chloride	0	0	0	0		0	0	0	0	0	0	0	0	0				
7	2.093	methylmethylether		0					0				0							
8	2.292	hexane	X	X	X				0	0			0		X					
9	2.442	diisopropyl ether	0	0	0	0		0	0	0		0	0		0	0				
10	2.676/2.809	2-butanone	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
11	3.026	tetrahydrofuran (THF) + chloroform		0					0				0							
12	3.243/3.610	3-methylbutanal	S		S		X	S							S				S	S
13	3.493	benzene + colum bleeding		0					0	0			0							
14	3.593	2,2,3,3-tetramethylbutane		0									0							
15	3.693/4.293	2-pentanone	X	0	X			X	X	X					X	X	X	X	X	X
16	3.693/3.776/4.360	3-methyl-1-butylamine				X	X													
17	3.693/4.360/4.393	pentanal	S	S	S			S	S	S	S	S	S	S	S	S	S	S	S	S
18	3.726	2-methyl-butanol								X										
19	3.776	heptane											X							
20	3.793	?	X																	
21	4.093	trichloroethylene		0					0	0			0							
22	4.260	s-methyl ester ethanethioic acid + 2-pentanone							X	X										
23	4.443	5-hydroxy-4-methyl-6-hepten-3-one		X																
24	4.460/5.893	1-methoxyethanethiol	X	X	X			X	X	X	X	X	X	X	X	X				
25	4.576	bromodichlore-methane		0	S				0	0			0							
26	4.610	1-pentene	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X
27	4.843	hexanal		X					X	X			X							
28	5.076	dimethyl-disulfide					X								X		X	X		X
29	5.410	toluene	0	X	X			0	0	0	0	0	X	0	X	0	X	X	X	X

No.	Retention time	Name	Pol	Ner	Flo	Nap	KoIM		Lin	Fif	Len	Fie	Gur	Nan	Fio	Nat	Npo	Nel	Net	Lil	Nin
30	5.860	2-heptanone								X				X							
31	5.926/8.277	heptanal	X	X	X				X	X	X	X	X	X	X	X	X		X	X	X
32	6.043	3-hexanone	X	X	X								X								
33	6.043	tetrachloroethylene		0							0			0							
34	6.110	cyclohexanone	0	0	0				0	0	0	0	0	0	0	0	0				
35	6.343	dibromochloromethane + hexanal												0							
36	6.343	hexanal (with contam.)	S	S	S				S	S	S	S	S	S	S	S	S	S	S	S	S
37	6.610	2-pentylfuran	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X
38	6.777/9.777	benzaldehyde	0	X	X				0	X	X	0	0	X	0	X	0	X	X	X	X
39	6.943/10.194/10.228	octanal	X	X	X				X	X	X	X	X	X	X	X	X				
40	7.094	chlorobenzene		0										0							
41	7.277	ethylbenzene		0										0							
42	7.427	p-xylene		0						0	0			0							
43	7.627	1-octanal			X					X				X							
44	7.727/7.760	4-heptanone					X						X	X							
45	7.744	2-n-butyl-furan	X	X	X				X	X	X				X						
46	7.894/11.995/12.045	nonanal	X	X	X				X	X	X		X	X	X	X	X		X	X	X
47	7.927	3-methyl-1-pentanol	X	X	X				X	X			X								
48	7.960	o-xylene + styrene		0										0							
49	8.177	tribromomethane + 2-heptanone		0										0							
50	8.177	2-heptanone (or methyl-ketone)	S	S	S		X		S	S	S		S	S	S	S	S	S	S	S	S
51	8.277	1,2-cyclooctanediene + ?	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X
52	8.961/10.211	toluene?	X	X	X				X	X	X	X	X		X	X		X	X	X	X
53	9.861	2,3-octanedione + benzaldehyde														S					
54	9.911	1,3-octadiene + branched C9 alkane		X					X	X				X	X						
55	10.961	3-ethyl-2-methyl-1,3-hexadiene			X				X	X				X	X	X		X		X	X
56	11.211	alkane									X		X			X	X				
57	11.245	2,2-dimethyl-3-hexanone									X										
58	11.511	(E)-2-octenal	S	S	S				S	S			S		S						
59	11.811	1-phenyl-1-pentanone(C11H14O isomer)		X						X		X	X								
60	12.045	nonanal + methylbenzoate	X	X	X				X			X	X								
61	12.912	dodecane			X						X		X			X	X	X	X	X	X
62	13.295	diethylbenzene	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X
63	13.528	?	S	S	S				S	S	S	S	S	S	S	S	S	S	S	S	S
64	13.778	O-isopropenyltoluene(C10H12 isomer)	S	S	S				S	S	S	S	S	S	S	S	S	S	S	S	S
65	14.495	alkane									X		X			X	X	X		X	X
66	16.796	1,2-dimethoxy-4-(2-propenyl)-benzene	S	S	S				S	S	S	S	S	S	S	S	S	S	S	S	S

No.	Retention time	Name	Pol	Ner	Flo	Nap	KoIM		Lin	Fif	Len	Fie	Gur	Nan	Fio	Nat	Npo	Nel	Net	Lil	Nin
67	18.162	1,1'-(1,3-phenylene)bis-ethanone	S							S					S	S					
68	18.179	dimethyl phthalate									X						X				
69	18.396	butylated hydroxytoluene	0																		
70	18.546	1,4-dihydro,-1,4-ethenonaphthalate				X															
71	20.947	diethyl phthalate									X		X			X	X				
Number of samples analyzed:			4	4	5	2	3		3	4	3	1	3	2	2	7	1	5	4	4	4
Number of compounds (excluding controls and sawdust):			17	21	23	4	8		16	23	25	11	19	19	13	22	16	15	15	16	17

Table 3.3: Further compounds were found in 2 additional lion samples. One sample from a GIV male cub (SG), and one sample from the bladder of a subadult KBH female (KB2).

No.	Retention time	Name	Found in sample:																		
72	1.592	azetidine?	KB2																		
73	2.993	(+/-)-2-butanol	KB2																		
74	5.393	3-methyl-1-butanol	KB2																		
75	5.743	3-methyl-pentane	KB2																		
76	7.260	4-methyl-3-penten-2-one	KB2																		
77	9.427	6-methyl-2-heptanone	KB2																		
78	10.044	2-octanone	SG																		
79	11.278	phenol	KB2																		
80	11.828	2-undecanone	SG																		
81	13.245	?(heptanal?+?)	KB2																		

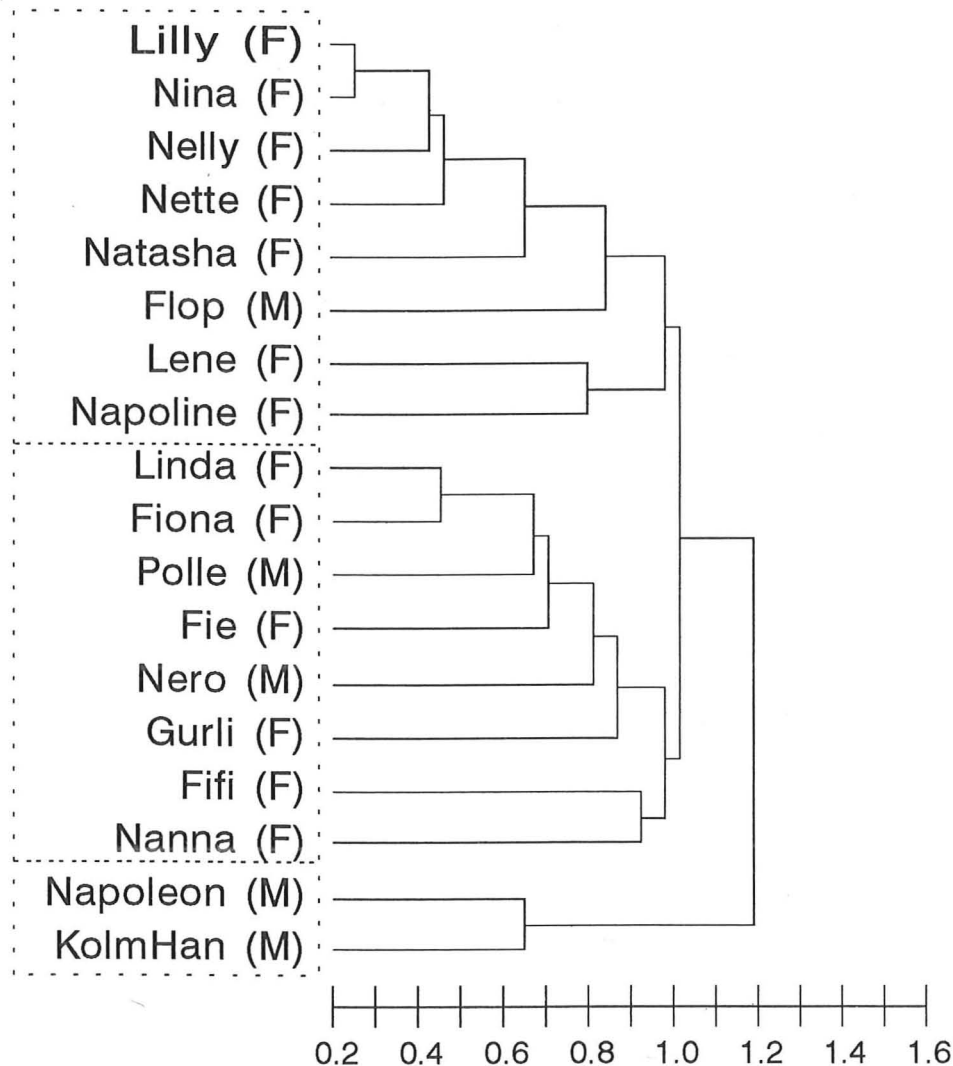


Figure 3.29: Average Linkage Cluster dendrogram showing the "relatedness" between individual lions of the chemical composition of scent marks. The axis at the bottom shows the root-mean-square distance between observations.

Note that three main groups are evident (indicated by the dashed boxes), and that the groups are not divided by sex. The two upper groups are all individuals from GIV. The bottom group (Napoleon and KolmHan) comprise individuals from two other zoos.

M: male, F: female.

Figure 3.30: Differences in compound overlap within and between the lion sex groups.

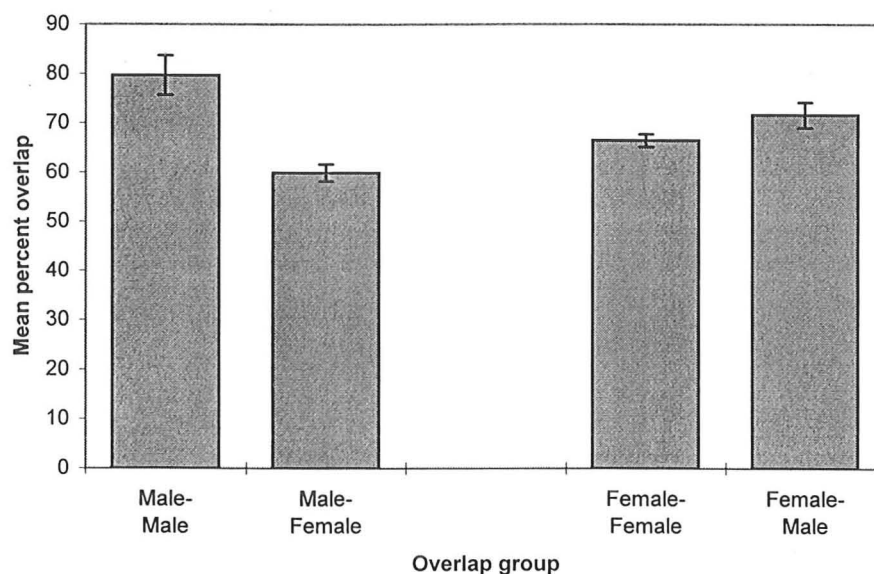


Figure 3.31: Percentage overlap in compound composition within and between individuals of the same sex groups for lions. Males are shown to the left and females to the right.

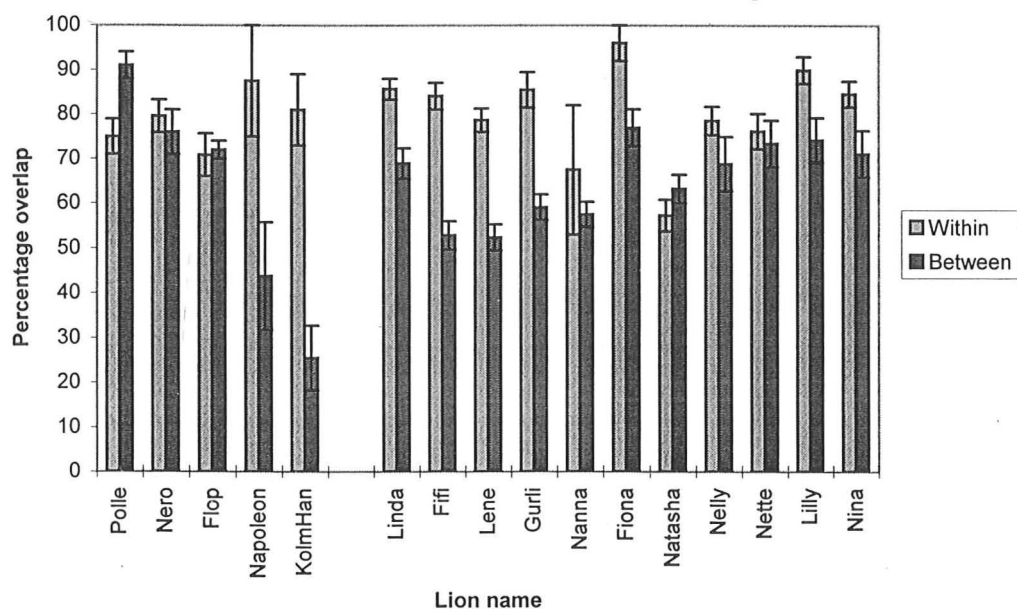


Figure 3.30: Differences in compound overlap within and between the lion sex groups.

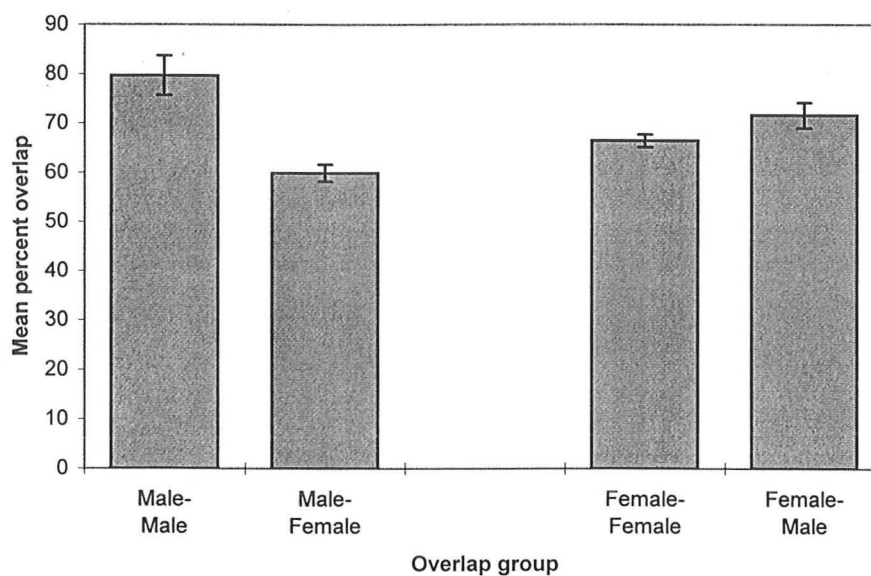


Figure 3.31: Percentage overlap in compound composition within and between individuals of the same sex groups for lions. Males are shown to the left and females to the right.

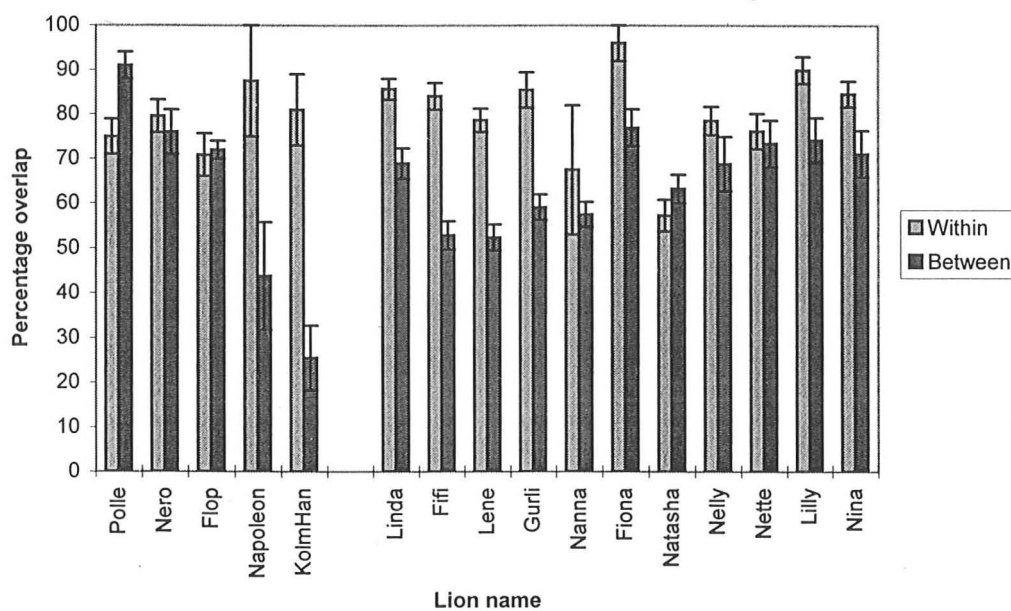


Figure 3.32: Sex difference in absolute concentration (area under the peaks of the chromatogram) for different compounds in lion urine.

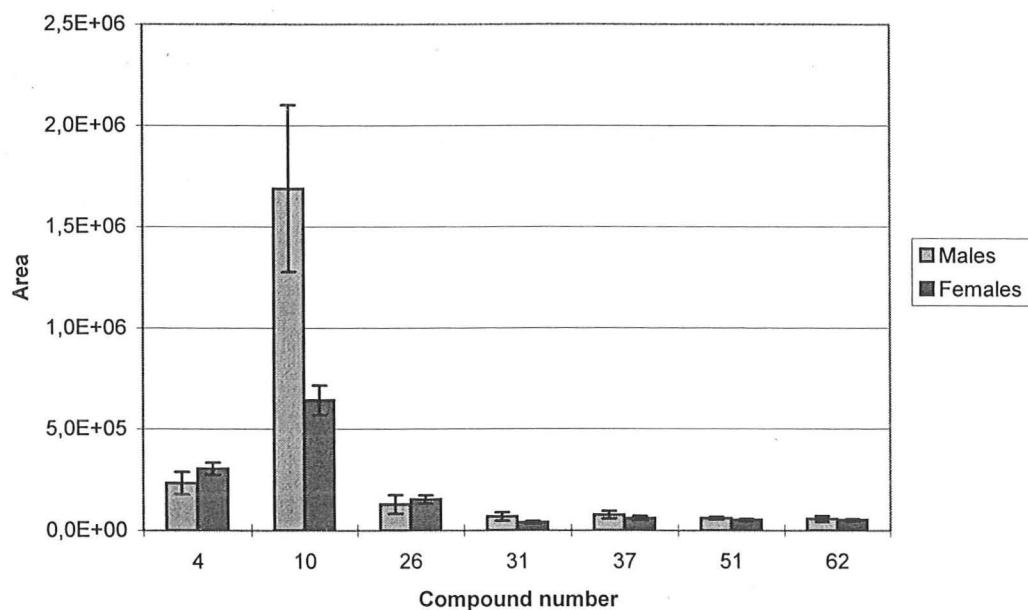
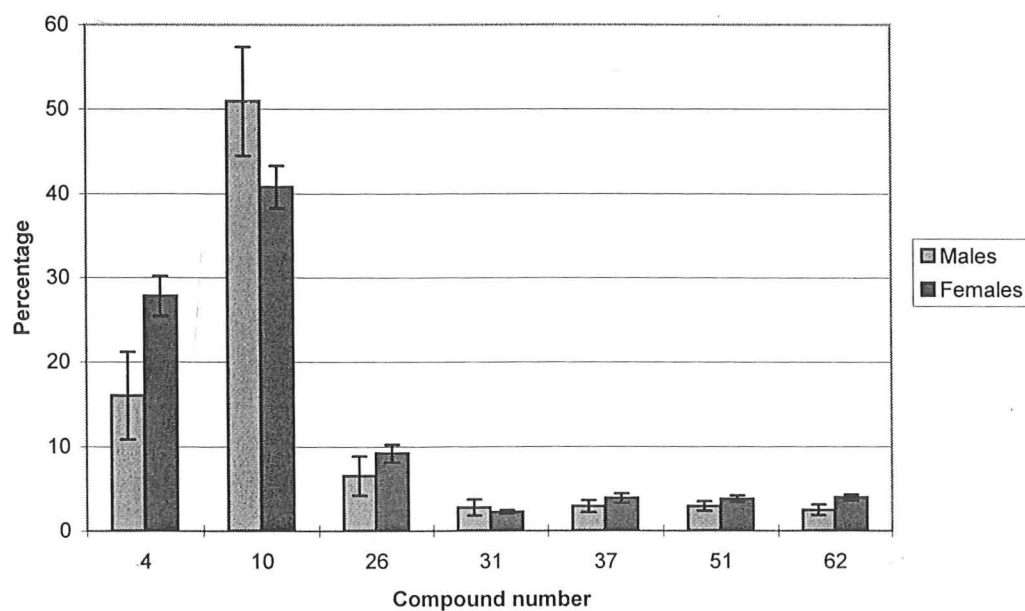


Figure 3.33: Sex difference in percentage concentration of different compounds in lion urine.





CHAPTER 4: OTHER *PANTHERA* SPECIES.

In addition to the lion, two of the other *Panthera* species were also studied. These were the Siberian tiger, *P. tigris altaica*, and the leopard, *P. pardus*. Being largely solitary animals the number of individuals available for study in captivity is less than that of lions leading to a smaller sample size. The results obtained from these two species are therefore more tentative than those for lions.

In this chapter the results on activity, social behaviour, scent marking behaviour and chemical analysis of urine from tigers and leopards are presented and discussed. The data on activity and social behaviours are baseline data which are used in evaluating whether the focal groups of each species are behaving similarly to wild populations. The data on scent marking behaviour and the chemical profile of the urine are used to test the hypotheses described in the Introduction chapter. The figures and tables referred to in the text are found between pages 140 and 161.

4.1 THE SIBERIAN TIGER: *Panthera tigris altaica*.

As pointed out in the Introduction chapter the tiger is a highly solitary species with each sex inhabiting exclusive territories with no normal access allowed for individuals of the same sex. Therefore tigers are usually kept only in pairs in captivity, as larger groups would almost inevitably lead to serious aggression. The only relatively safe way in which a large group can be kept in the same enclosure is by castrating all, or all but one, of the adult males and letting the females out one at a time.

In Copenhagen Zoo (KBH) a pair of tigers was kept. The male was named Sym and the female Mercedes. In Knuthenborg Safaripark (KNU) the tigers were kept as a group in which all but one of the males were castrates, and the females were kept apart so that only one was allowed into the main outdoor enclosure at any one time. The names of the intact adult male tiger was Amur. The two castrated adult males were called Jahrin and Amba. Three females were present and they were named Majenka, Pepejja and Unda. Four male cubs which had also been castrated were also part of the group and they were called Kacug, Abakajn, Sibir and Ruslan. Kacug and Abakajn were 1½ years old when the fieldwork started, and Sibir and Ruslan were 1 year old. In the results and discussion sections in this chapter abbreviations for the four sex/age groups will be used in the

following way: Intact male = M, Castrated males = MC, Females = F and Castrated cubs = CC. The one male in KNU that was intact at the beginning of the study was also castrated, due to aggression towards the other tigers, just before the fieldwork was about to begin, leaving the KBH male as the only intact male in the study groups. A sample size of one is too low with quantitative data, as is the case in parts of this chapter, so the results can only be suggestive. On the other hand the KBH male could well be typical of the average Siberian male tiger. The presence of the three adult male castrates in the KNU group provides an opportunity to identify possible effects of this operation on the scent marking behaviour of tigers in particular and *Panthera* species in general.

4.1.1 Activity.

The observations of the activity of tigers are used for comparison with the behaviour of wild tigers to see if the activity is affected by captivity. If the general behaviour is similar it may be expected that the marking behaviour of captive tigers is similar to that of wild animals.

Results.

The activities of the individual tigers at both KBH and KNU are shown in **Figure 4.1.1**. The main activities for all tigers were *Lying* (Mean= 69.4% \pm 7.9) and *Moving* (Mean= 25% \pm 8.2), with *Standing* (Mean= 2.8% \pm 1.9), *Other* (Mean= 2.5% \pm 1.2) and *Sitting* (Mean= 0.4% \pm 1.4) occurring at lower frequencies. For the statistical analysis of differences between the sex/age groups the data were divided into the following four groups: male, castrates, females and cubs. There was a significant overall difference between the activities of the tiger sex/age groups ($\chi^2=905.8$, DF=12, $P<0.001$), and this difference is specified in the next figure. In **Figure 4.1.2** the contribution of each cell in the Chi-square test to the overall Chi-square value is shown for each of the four sex/age groups. The direction of each deviation is also included to clarify if a particular sex/age group is over represented (positive value) or under represented (negative value) relative to the other groups with regards to the particular activity categories. The main contribution to the overall Chi-square value comes from relative high level of male *Sitting* in KBH. Other important contributions come from the high level of female *Moving* and the low level of cubs *Moving*.

Discussion.

As tigers are almost impossible to follow visually for any length of time in the wild, all the activity studies reported in the literature have been carried out by radio tracking, and they are therefore able to determine only whether an animal is active or not, and not the nature of the activity. The general picture of tiger behaviour is one of predominantly nocturnal activity, but with recurrent periods of movement taking place during the daytime (Schaller 1967; Seidensticker 1976; Matjushkin *et al.* 1980; Sunquist 1981). As none of the zoos were willing to permit access at night time the observations during this study were confined to the daylight period, and it is therefore impossible to say whether the animals were more active during the night. Considerable activity was seen during the day, and the timing and duration of this activity was to a great extent determined by the routines of the zoos and the social environment of the animals. Exner (1995) provides comparable results from captive tigers in Germany. She found that the animals were *Moving* 34% of the time, *Sitting* 1% and *Lying* 60% of the time. In 5% of observations the animals were out of sight. These figures are of a level comparable to those found for the KBH and the KNU tigers.

The main difference in activity between the tigers of this study and wild tigers appears to be the shift from night time to daytime activity. This shift in itself does not necessarily lead to changes in other aspects of the tigers' behaviours. As far as the activity is concerned there are no indications that major deviations from normal levels occur among the tigers in this study, but without firm data on wild tigers it is difficult to say with certainty.

4.1.2 Social behaviour.

The results presented on tiger social behaviour are also part of the baseline data used in the evaluation of any effects which abnormal natures or levels of behaviours might have on the scent marking of the animals.

Results.

The first results presented on the social behaviour of the tigers are produced by a qualitative analysis in which figures are drawn showing the direction of social interactions between the individuals within each group. **Figure 4.1.3** and **Figure 4.1.4** show the directions of social interactions observed between the KBH tigers and the KNU tigers respectively. Please refer to page 30 in Chapter 2 for a definition of the behavioural categories.

Headrubbing: In KBH *Headrubbing* was directed by both the male and the female towards each other. In KNU there was very little *Headrubbing* between the males and the females. Amur (MC) and Jahrin (MC) were the main targets. It was performed by the cubs towards only their mother and not other females.

Bodyrubbing: In KBH the female was the actor in *Bodyrubbing*. In KNU it was not observed at all.

Allogrooming: Mutual *Allogrooming* was shown by the male and female in KBH, whereas in KNU it was most pronounced between the adult males, between the males and the cubs and within each of the two litters.

Playing: All individuals in both groups were involved in *Playing* behaviour. In KBH it occurred in both individuals and in KNU it was also widespread throughout the group, except that the females very rarely indulged in it. The three adult castrated males interacted mainly with each other. The cubs interacted mainly among themselves, but also directed *Playing* towards the males and females, both mother and non-mother.

Investigation: *Investigation* was mutual in the KBH group, but in the KNU group the males, Amur (MC) and Jahrin (MC), were the main targets of this behaviour. The only female targeted for *Investigation* in KNU was Unda (F), who at that time was in oestrus.

Mild Aggression: *Mild Aggression* was widespread in the groups. Both individuals in KBH were actors in this behaviour, and in KNU all individuals were both actors and targets of *Mild Aggression*, though Amur (MC), Majenka (F) and Pepejja (F) were not targeted by as many of the other tigers as the rest of the individuals.

Serious Aggression: *Serious Aggression* was quite rare. It was performed by both tigers in KBH, but in KNU it was largely centred around one individual, namely Abakajn (CC) who interacted in this way with more of the other individuals than the rest.

Mating: No *Mating* was observed in KBH. In KNU it was seen only between Amur (MC) and Unda (F) even though Amur had been castrated some months previously. Mock mating was observed between the three adult male castrates in KNU.

Moving on to the percentage distribution of the six most frequently observed social behaviours, the results are presented in **Figure 4.1.5** for KBH and KNU tigers. It is clear that there are big differences between most of the individuals, with the most eye-catching being the difference between the KBH female (Mercedes) and the three KNU females (Majenka, Pepejza and Unda). The three KNU females are very similar in their social repertoire as are the four KNU cubs. A Chi-square test for overall differences between the four sex/age groups (male, castrates, females and cubs) show that a significant difference is present ($\chi^2=540,6$, $DF=15$, $P<0.001$).

The overall sex/age group difference can be broken down to show the contribution of each of the individual cells in the Chi-square test. These contributions are shown in **Figure 4.1.6** including an indication of whether the deviation is relatively higher (positive value) or lower (negative value) compared to the other sex/age groups. The group which contribute most to the overall Chi-square value is the cub group with its high level of *Playing*, low level of performing *Headrub* and low level of *Mild Aggression*. The castrates group contribute with high level of *Headrub* and *Allogroom*, and a low level of *Playing*. The females have a low level of *Playing* and a high level of *Mild Aggression*.

The final step in the analysis of the social relationships between the tigers in the study groups is to analyse the amount and direction of *Non-Aggressive* and *Aggressive* behaviours received by each of the individuals or sex/age groups. The *Non-Aggressive* categories for which the number of interactions were summed were *Headrubbing*, *Bodyrubbing*, *Allogrooming*, *Playing* and *Investigation*, and the *Aggressive* categories were *Mild Aggression* and *Serious Aggression*. The figures show the targeted individual or sex/age group (the "vs." category typed in bold under the x-axis) and the individual or sex/age group that acted towards them (the normal typing just below the x-axis).

There was no significant difference in KBH between the *Non-Aggressive* and *Aggressive* patterns of the male Sym and the female Mercedes, **Figure 4.1.7** (χ^2 -test, NS).

In KNU, **Figure 4.1.8**, each of the three adult male castrates are depicted separately. The general picture of male towards male interactions is one of predominantly *Non-Aggressive* and few *Aggressive* interactions. However, *Non-Aggressive* interactions are not distributed evenly among the males. Jahrin (MC) receives by far the major part of the *Non-Aggressive* interactions observed.

The females generally interact *Aggressively* with the males, though Amur (MC) received equal amounts of *Non-Aggression* and *Aggression* from them.

The predominant behaviour of the cubs towards the males was *Non-Aggressive*, but

Aggression was also seen though at very low levels.

The pattern of received *Non-Aggression* and *Aggression* from the different sex/age groups were significantly different for all three males (χ^2 -test, DF=3, $P < 0.001$).

The females received almost equal amounts of *Non-Aggression* and *Aggression* from the males, and more *Non-Aggression* than *Aggression* from the cubs. The overall difference between interactions that the females received from males and cubs was not significantly different ($\chi^2 = 1.0$, DF=1, $P = 0.323$).

Aggression was the dominant action towards the cubs by both males and females, whereas the interactions between the cubs were mainly *Non-Aggressive*. The patterns received by the cubs from males, females and cubs were significantly different ($\chi^2 = 267.2$, DF=2, $P < 0.001$).

Discussion.

The social environment of the tigers in the zoos is very different from that of wild tigers. The wild tiger is a highly solitary and territorial animal which usually avoids encounters with conspecifics of either sex (Schaller 1967; Seidensticker 1976; Matjushkin *et al.* 1980; Sunquist 1981). As a consequence the only regular social activities seen among wild tigers are between a female and her dependent cubs, but unfortunately no quantitative data for such interactions are available. Incidents of mutual tolerance between two adult tigers feeding at the same kill has been reported (McDougal 1977), but most of these were at artificial baiting sites, which makes them more or less as unnatural as the zoo environment. These interactions were always reported to be dominated by aggressive behaviour and no directly amiable social interactions between two wild adult tigers has been reported.

Only when females have dependent young or when mating is taking place are any lengthy associations seen between individuals. Therefore the level of social interactions between the tigers in captivity will be much higher than that which would occur among wild tigers. The true nature of social interactions between wild tigers is reflected in the most common group composition for tigers in captivity, namely one adult male and one adult female, thus avoiding the otherwise very likely occurrence of intra-sex aggressions. As mentioned previously this was also reflected in the management policy in KNU in that all three adult males were eventually castrated to minimise agonistic behaviour, and only one adult female was let out into the main enclosure at a time. The four cubs were also castrated by the Park veterinarian to prevent future aggression in the group. Castration, however, does not remove the risk of serious aggressive behaviour completely. This was

demonstrated in KNU cubs where particularly the big cub Abakajn, on the point of being subadult, started to show aggressive tendencies towards the other tigers, and especially the females. His attacks led to a few incidents of serious fighting and injury, and encouraged Amba, and to a lesser extent Amur, to participate in the fighting. The park staff had to be very vigilant and determined in their interventions to prevent the fighting from escalating, but some months after the completion of the fieldwork, the group had to be split up and individuals had to take turns in using the main enclosure.

The KBH female Mercedes performed most of the *Headrubbing* seen at this location. The most frequently observed behaviour for the castrated KNU males was *Headrubbing* which was performed mostly among themselves. Even though friendly encounters between wild tigers are rare *Headrubbing* still seem to function as a greeting and a friendly gesture. Considering the normal aggressive interactions of wild tigers the function of *Headrubbing* seems a bit odd. The reason why this behaviour is retained in their repertoire could be threefold. Firstly, it could be because cubs practice this behaviour as an appeasement towards their mother and therefore adult tigers remember this function. Secondly, it might be an old innate behaviour left over from a time when tigers were more social. Thirdly, in areas of high tiger density or when conditions are adverse and prey scarce and patchy tigers have been reported to form loose associations for a period (Bragin 1986) and in these situations *Headrubbing* may be an important social regulator.

The tiger pair in KBH had only each other to interact with. All interactions were performed by both animals towards the other except *Bodyrubbing* which was performed by the female, Mercedes, only. Whether *Bodyrubbing* may be a precursor for full mating behaviour in the KBH female is not known, but as it was not observed for the KNU female which came into oestrus and mated with one of the males this seems unlikely.

The function of *Allogrooming* in tigers is probably both hygienic and social. However, due to the social system of tigers it is not very widespread and occurs almost exclusively between mothers and cubs. Whether it also might be part of the normal mating behaviour of tigers remains to be seen.

Playing among adult wild tigers has never been documented, but among cubs it is common. The *Playing* which was observed between the adult castrated males in KNU is probably a result of an increase in their social tolerance caused by the castration.

A substantial part of the *Investigation* observed in KNU was directed towards the castrated male Amur. As he was castrated just before the start of the observation periods, the reason for the great interest in him could have been the castration which apart from a physical scar, would probably also lead to changes in his odour and urine composition (see results of castration under sections Scent making and Chemical analysis. The only female

in KNU to be targeted for *Investigation* was Unda which at that time was in oestrus.

The two tigers in KBH showed but *Mild* and *Serious Aggression* towards each other, but it never resulted in actual bodily injury. This, however, did happen in KNU. All individuals showed some level of Mild Aggression and the cubs were the most heavily targeted group. This was probably because they were physically inferior to the other individuals (though almost comparable to the females in body size) and they often tried to initiate playing with the adult individuals which frequently responded with threats. The cubs also sought proximity to the females and especially their respective mothers, but again they were met with threats. *Serious Aggression* was also seen in KNU and in one instance it resulted in a serious body wound on one of the females. The big cub Abakajn was the main instigator of *Serious Aggression* in the group, and the main targets were the non-mother females Pepejja and Unda. Why he showed this level of aggression towards the females is not known, but apparently his castration had not had the desired effect of eliminating serious conflicts in the group.

Even though the adult male Amur had been castrated he still responded with normal mating behaviour when approached by a female in oestrus as was seen when the female Unda came into oestrus. Whether the frequency of mating was lower than would be expected for an intact male is difficult to say as no data are available on this issue. As for the intact male lions, mock mating was observed between the adult castrated male tigers, but not between any of the other sex/age groups. The function of mock mating, if any, is not clear, but it could be seen as an aberrant consequence of captivity.

There was a big difference in the social repertoire of the three KNU females and the KBH female. The KNU females' repertoires were dominated by Mild Aggression whereas the KBH female had a very high percentage of Headrubbing. This difference could well be related to the difference in social grouping between the two locations, but one would have to relocate individuals or change group structures to confirm this suspicion.

The pattern of Non-Aggressive and Aggressive behaviours showed by the two KBH tigers towards each other was most similar to that seen among the cubs in KNU. The three KNU males generally showed more Non-Aggressive than Aggressive behaviours towards each other, whereas they showed more Aggressive than Non-Aggressive towards the cubs. It seems likely that the amiable social atmosphere among the adult males is a consequence of their castration, but without pre-castration observations of the same animals one cannot be sure. Very few interactions were directed by males towards females. The females showed more Aggressive than Non-Aggressive behaviours towards both the adult males and the cubs, thus demonstrating their wish to be left alone. The cubs showed more Non-Aggression than Aggression towards all other groups, suggesting that they are still too

young to show any interest in territorial behaviour.

Again it must be stressed that the management of captive tigers results in a group structure which is very far from that seen in wild tigers, and therefore the number and nature of social interactions are likely to be influenced by this factor. Whether it will have any effect on the types and rates of scent marking observed is more difficult to say. It is quite likely that the females in KNU exhibited higher levels of scent marking than would have been seen if they had been the only female present. Each time a female tiger was allowed into the main enclosure she would encounter the scent marks deposited by the female who was there the day before, so they always encountered fresh foreign scent marks. In addition the two female tigers which were not in the main enclosure were kept in smaller enclosures situated next to the building containing the night cages and in full view of the other tigers and only separated from the main enclosure by a wire mesh fence. Pepezja and Unda in particular would frequently pace back and forth on each side of the fence growling and *Spraymarking* frequently. This issue will be discussed further in the following section on scent marking.

4.1.3 Scent marking.

This section presents the results on the scent marking repertoire and scent marking rates of the undisturbed focal tiger groups. These observations were carried out to test Hypotheses 1 and 3 (page 19) by looking for differences in marking behaviour between the sexes and the effect which castration might have on this behaviour. Included are also the results of the scent presentation experiments which were conducted to test Hypotheses 4, 6, 7 and 8 (pages 19- 20) by investigating the animals' ability to distinguish between individual scent marks and between different categories of scent marks.

In all experiments distilled water was used as a neutral control mark. As no reaction was ever observed towards the water marks they have been omitted from the following analysis.

Results.

Scent marking repertoire.

The first analysis carried out in order to investigate the scent marking behaviour of the two tiger populations was to compare the percentage distribution for marking types

observed for each of the individuals in KBH and KNU. These results are shown in **Figure 4.1.9**. Two of the individuals in KNU were never observed to *Spraymark*, namely the castrated male Jahrin and the female Majenka. Apart from these two, all the other adults, including the other two castrates, had *Spraymarking* as the main type of scent marking. No significant differences were found with regards to the percentage of *Spraymarking* between the male and the castrates ($t=0.71$, $DF=2$, $P>0.05$), between the male and the females ($t=0.29$, $DF=3$, $P>0.05$), or between the castrates and the females ($t=0.62$, $DF=5$, $P>0.05$).

Faeces was usually deposited in a random fashion by the tigers, but on seven occasions two of the female tigers in KNU (Pepeszja and Unda) were seen to deposit faeces instead of urine on top of scrapes.

Scent marking rates.

The rates of marking for the five main mark types are shown in **Figure 4.1.10**. Four of the tigers had marking rates which were much higher than the rest. These were the KBH male Sym, the KBH female Mercedes and the two KNU females Pepeszja and Unda. For these four individuals *Spraymarking* was by far the most frequently deposited mark type. A comparison of the rate of *Spraymarking* between the adult sex groups revealed the following differences. The intact male had a significantly higher rate of *Spraymarking* than the three castrated males ($t=21.73$, $DF=2$, $P=0.002$), and even if the castrate with the zero-value is excluded the difference is still significant ($t=21.75$, $DF=1$, $P=0.029$). No significant difference was found between the *Spraymarking* rate of the intact male and the females ($t=0.11$, $DF=3$, $0.05 < P$), or between the castrates and the females despite the latter having a much higher mean rate of *Spraymarking* (unequal variance, $t=2.17$, $DF=5$, $P=0.082$). However, if the zero-value from each of the groups is excluded a significant difference is found between the castrates and the females ($t=4.02$, $DF=3$, $P=0.028$).

Further aspects of *Spraymarking* behaviour.

The two largest cubs in KNU, Kacug and Abakajn, showed a curious relationship between the number of *Spraymarkings* and *Urinations* observed and the identity of the female which was with them in the main enclosure on any particular day. This relationship

is depicted in **Figure 4.1.11** which show the number of *Spraymarking* and *Urination* observed and the identity of the female present. Both cubs had a significantly higher number of *Spraymarkings* compared to *Urinations* on days when they were together with their mother, Majenka, than on days when they were together with the non-mother female, Unda (Kacug: $\chi^2=14.94$, $DF=1$, $P<0.001$; Abakajn: $\chi^2=6.13$, $DF=1$, $P=0.013$).

The effect of castration on *Spraymarking* rate was observable in Amur, who was castrated one month before the fieldwork in KNU began. In **Figure 4.1.12** the rate of *Spraymarking* for Amur is depicted at two month intervals starting one month after the castration. It is clear that his rate of *Spraymarking* has fallen drastically and that there is no indication that it will rise again. Note that the readings at month "1" and "13" are taken at the same time of the year so that the decline illustrated by these two readings is actual and cannot be ascribed to any seasonal fluctuations.

Investigation of natural scent marks.

As explained in the previous chapter it was difficult to collect accurate data on reactions of the animals to scent marks deposited by other group members as the location of the marking sites could not be memorised for long by the observer. In KBH no reactions were observed, and in KNU only on 21 occasions could the donor of the mark as well as the reactor be accurately determined. Of these five were reactions to *Spraymarkings* (two towards castrate male marks and three towards female marks), three were reactions to *Scrape/urinations* (all three towards female marks) and the remaining 13 were reactions towards normal *Urinations* (all castrate male marks). Almost all of the reactions were *Scenting* and *Flehmen* whereas *Licking* and *Overmarking* were extremely rare.

Presentation and reaction to experimental scent marks.

In KBH results on the Indoor scent presentation experiments are available only for Sym, as Mercedes at the time of these experiments had died of an illness. In KNU an occurrence of serious aggression between some of the group members meant that I was not allowed to continue the Outdoor experimental work, and neither was I allowed to start

on the Indoor experimental work because of fear of a further escalation in aggression.

The first results presented in this section illustrates the qualitative nature of the tigers' responses i.e. whether there were any differences in the relative proportion of the marks reacted to for the different categories of experimental marks. These are shown in **Figure 4.1.13** as the cell chi-square values from the χ^2 -test including an indication of whether the response was positive or negative. Data are from the total combined KBH Outdoor and Indoor, and the KNU Outdoor experiments. None of the sex/age groups showed a significant difference in their proportion of reactions to the different types of marks, but some tendencies were present. The male showed a negative response to Tiger female marks and a positive one to Lion marks. The castrated males showed little interest in the Tiger male marks compared to the Lion marks. The females showed a slight preference towards Tiger male marks relative to the other categories. The castrated cubs exhibited the same pattern as the adult castrates but with less amplitude.

The responsiveness of the tigers to the experimental marks varied with the location of the experiments. In the Indoor experiments in KBH the male responded to 88% of the marks presented. In the Outdoor setting in KBH at least one of the tigers responded to 78% of the marks. In KNU only Outdoor experiments were conducted and here females responded to 50% of the experimental marks whereas the castrates responded to only 25%.

When investigating scent marks tigers exhibit four characteristic types of behaviour. They start by smelling the mark (*Scenting*) and this may be followed by one or more of the three other types which are *Flehmen*, *Licking* and *Overmarking*. *Licking* and *Overmarking* were observed very infrequently and the following analysis will therefore concentrate on *Scenting* and *Flehmen*. These two behaviours were quantified for each of the sex/age groups when they investigated different types of experimental marks, and the results are shown in **Figure 4.1.14** and **Figure 4.1.15** respectively. The results on *Scenting* behaviour (Figure 4.1.14) will be commented on first.

Male tiger: In the KBH Outdoor experiments the male tiger showed a significantly higher frequency of *Scenting* towards Tiger male marks than towards either Tiger female marks, Tiger female oestrous marks or Lion marks (all $P < 0.001$). A similar result was not seen for the KBH Indoor series.

Female tiger: No significant differences were found for either the KBH or the KNU females in their *Scenting* frequencies towards the different types of experimental marks.

Castrated male tiger: The castrated male tigers in KNU showed significantly more *Scenting* towards Lion marks than towards Tiger male marks.

Castrated cub tiger: No significant difference was found for this group.

Comparison between sex/age groups:

KBH Outdoor: The male had significantly higher frequencies of *Scenting* towards both Tiger male marks ($P < 0.001$) and Tiger female oestrous marks ($P = 0.010$) than the female.

KNU: The females showed significantly more frequent *Scenting* towards Tiger male marks than both the castrates and the cubs (both $P < 0.001$). Furthermore, both the females and the castrates had a higher frequency of *Scenting* towards Lion marks than the cubs (both $P < 0.001$).

The results for *Flehmen* (Figure 4.1.15) show very similar trends to the *Scenting* results but the differences are generally less pronounced.

Male tiger: No significant differences were found in the male tigers' *Flehmen* frequencies towards the different experimental scent marks in either the KBH Outdoor or Indoor series. A strong tendency of more *Flehmen* towards Tiger male marks was however present.

Female tiger: No significant difference was found between the KBH or KNU females in their investigation of the scent mark types.

Castrated male tiger: In KNU the castrates showed significantly more *Flehmen* towards Lion marks than towards Tiger male marks ($P < 0.001$).

Cub tiger: No significant difference was found for the cubs.

Comparison between sex/age groups:

KBH Outdoor: In KBH the male tiger showed significantly more *Flehmen* than the female towards Tiger male marks ($P < 0.001$), Tiger female marks ($P = 0.012$) and Tiger female oestrous marks ($P = 0.034$).

KNU: No significant differences were found in KNU between the sex/age groups..

Discrimination of scent from two individuals.

The results obtained from the Indoor experiments in KBH were used to test Hypothesis

4 (page 19) i.e. that tigers are able to discriminate between the scent marks of two foreign individuals. The investigations by the intact male Sym of the four simultaneously presented experimental marks were divided into investigations of the Odd mark and the three Like marks (Like 1, 2 and 3) according to the order in which they were investigated. The results for this analysis are given in **Figure 4.1.16**. There was no significant difference between the mean time he spent in investigation of the Odd mark and the Like 1 mark ($t=0.02$, $DF=56$, $0.05 < P$), but there was a significant difference between his levels of investigation of Like 1 and Like 2 (unequal variance, $t=2.47$, $DF=37.6$, $P=0.018$), and Like 2 and Like 3 (unequal variance, $t=2.74$, $DF=44.2$, $P=0.009$).

Discussion.

The scent marking observed in the captive animals in this study was similar to that reported for wild populations. Scent marking in the wild tiger has been described by several authors (Schaller 1967, McDougal 1977). Both noted that tigers of both sexes scent mark by use of urine spraying, by scraping, sometimes in combination with urination or defaecation, and by the clawing of trees. The *Spraymarking* behaviour is first seen in cubs of two to three years of age (Choudhury 1980; Brahmachary and Dutta 1981). Choudhury (1980) found considerable variation in the number of scent marks deposited by his female pet tiger on consecutive days, and he suspected that a seasonal variation was present. Both Schaller (1967) and McDougal (1977) reported that oestrous females marked with increased intensity, but this is in direct contrast to Choudhury (1980), Smith *et al.* 1989 and Poddar-Sarkar *et al.* 1995 who all found that marking rates for females increases up to oestrus, but during oestrus very little marking is seen. Smith *et al.* (1987) observed that territorial female tigers marked at a higher frequency along the territorial borders than within. It has also been reported that individuals of either sex which are in the process of establishing themselves as territory holders mark at much higher frequencies than those with established territories (Sunkist 1981; Smith *et al.* 1989; Smith 1993). Both Schaller (1967) and McDougal (1977) found that tigers marked with both *Spraymarkings* and *Scrape/urinations* when moving along trails within their territory, but McDougal noted that Scrapes were most frequent in the core area of the territory.

No information on the relative frequency of the different marking types are available from studies in the wild, so it is not possible to say whether the frequency of mark types or the rate of marking observed in KBH and KNU are comparable to those of wild tigers.

The KNU male patterns are particularly unlikely to resemble those of wild tigers as all the males were castrates. Except for the three KNU females very little *Scrape/urination* was observed for the tigers. This almost complete lack of *Scrape/urination* for most of the individuals supports that either this behaviour develops later than *Spraymarking* behaviour, which would explain why it is also absent in KBH, or it disappears when an animal is castrated. Probably a combination of these two factors, i.e. that it does develop later than *Spraymarking* behaviour and that it is under some level of hormonal control, is the most likely explanation. One further possibility is that *Scrape/urination* is closely linked to the marking behaviour of only established territory-holding tigers, and that the castration of an animal destroys its sense of territoriality thereby reducing or removing *Scrape/urination* from its marking repertoire. In this case the lack of *Scrape/urination* among the two young tigers in KBH could be seen as an indication that they do not yet see themselves as properly established in the "territory" provided by the KBH enclosure.

The complete lack of *Spraymarking* in the repertoire of the KNU female Majenka is puzzling, but as she seemed to be socially subordinate to the other females as well as the adult males, it might be a question of social inhibition.

No difference was found in the percentage of *Spraymarking* in the repertoire between males, castrates and females. It thus appears that castration does not influence the relative amount of *Spraymarking*. The oldest of the males in KNU, Jahrin, had ceased to scent mark altogether, but this was probably an age related phenomenon rather than an effect of the castration. He died of age related conditions shortly after the fieldwork ended in KNU.

That faeces may play a role in the scent marking system of the tiger is indicated by the fact that on seven occasions female tigers were seen to deposit faeces instead of urine on top of scrapes. Similar observations has been made by Schaller (1967), McDougal (1977) and Sunquist (1981). Why faeces are sometimes deposited instead of urine is not clear, but it is likely that faeces retains its scent for a longer period of time than urine, so possibly faeces could be used in instances where a long-term mark is needed, but at present this point is purely speculative. It might be that defaecation is a process which the animals cannot regulate voluntarily and therefore only those scrapes made at the time of an approaching natural defaecation will be topped off with faeces. There are no indications that the scrapes on which faeces are deposited are distributed in a different way from those marked by urine.

The rates of scent marking showed major differences between the intact male and the castrates. *Spraymarking* was significantly higher for the intact male. This is evidence in support of Hypothesis 3. Further support for this Hypothesis was found in the declining *Spraymarking* rate seen for Amur after he had been castrated. There was no difference in

the *Spraymarking* rates of male and females, and this apparently contradicts the predictions of Hypothesis 1, namely that males hold larger territories than females and should therefore have higher rates of marking than females. However, it might be that since tiger males and females both hold individual territories, both sexes use scent marking to such an extent that their rates become comparable. Indeed scent marking with urine (*Spraymarking* and *Urination*) is based on a limited resource (see paragraph below), namely the urine, and this only allows the animal to reach a certain rate of marking. Therefore, once this limit in scent marking rate is reached an increase in the territory of the animal can not lead to an even higher rate of scent marking, and assuming that both the male and female territory are big enough for this upper limit to be reached, one would expect to find comparable marking rates for the two sexes. Unfortunately no quantitative data are available on scent marking rates for wild tigers.

That urine is a limiting factor in scent marking has been noted by Choudhury (1980), and it was also evident from the behaviour of the tigers in both KBH and KNU. On days when the *Spraymarking* rate was very high it was noted on several occasions that the tiger would run out of urine. This would not prevent it from keeping on adopting the characteristic *Spraymarking* posture, but despite this effort no fluid would be ejected from the urinary tract. The tiger did not seem to be aware of this fact and would keep on "*Spraymarking*". If this is indeed the case the behaviour described for tigers of turning around and inspecting the newly marked site (Schaller 1967, McDougal 1977) could be a way of making sure that urine had actually been deposited. The phenomenon of running out of urine has not so far been described for wild tigers which makes it possible that it could be related to the captive environment where animals may be stimulated to mark at higher rates than in the wild.

Social facilitation and inhibition may be factors influencing the scent marking behaviour of tigers. This was indicated by several factors in KNU. Majenka, the female which appeared to be socially subordinate to the rest of the adults, performed no *Spraymarking* at all, whereas the other two females, probably provoked by frequent encounters along the partitioning fence, showed very high rates of *Spraymarking*. Another indication of social facilitation/inhibition was seen for the cubs. Cubs may be more socially confident when together with their mother rather than when with another female. This confidence could be reflected in their marking behaviour. The two large cubs in KNU, Kacug and Abakajn, showed significantly higher ratios of *Spraymarking* to normal *Urination* when they were together with their mother (Majenka) than when they were together with another female (Unda).

Three brief accounts of scent experiments involving tigers were found in the literature.

In the first, Van den Brink (1977) presented a group of three captive tigers with the secretion ("Sekret") from a civet cat (*Viverra civetta*). The exact origin of the secretion and the circumstances under which it was obtained are not specified. The presentation was made in the outside enclosure of the tigers on four marking posts already used by them. The author found no significant increase in marking of these particular spots. The second experiment was carried out by Choudhury in India. He presented his female pet tiger with a twig on which a male tiger had previously marked, and he compared her reaction to that towards another twig on which female tiger urine had been applied. The reaction to the first stimulus was much longer and more intense (more *Flehmen* and *Licking*) than to the second (Choudhury 1980). In the third experiment, Whittle (1981) carried out a short series of scent presentations in the night cages of the tiger group at Windsor Safaripark. At that time eight tigers (four males and four females) were kept at Windsor. He obtained scent marks from most of these tigers by lining a cage with Whatmans Benchkote paper and collecting it after it had been marked by one of the tigers. This paper was then presented to the tigers (including the donor) by propping it against the bars of a tiger cage with a forked pole. If his presentation results are reconstructed into a form similar to the one used in this study, his results can be summarised in the following way. Male tigers responded more than expected to marks from the other males, less than expected to marks from the females and as expected to marks from oestrous females. Female tigers reacted as expected towards male tiger marks, less than expected to female tiger marks and more than expected to marks from oestrous female tigers. There are two important differences in the methodology used by Whittle and the one described in this study. Firstly the presentation of the marks is different in that the propping up of the paper against the bars using a pole creates considerable disturbance which may attract the attention of the tigers towards the marks. In my study the scent marks were presented by spraying them onto the wall of the night cage when the tiger was in the outside enclosure. The second difference is that Whittle used only scent marks which he obtained from individuals within the group to which he also presented the scents. Hence the tigers only encountered scent from individuals with which they were already familiar. In this study only scent from completely foreign individuals was used.

Some of Whittle's findings were reflected in the results of the present study. In both studies male tigers responded more to marks from other male tigers than to female marks. Though only a few of the differences in responsiveness shown by the tigers in this study towards the various categories of experimental marks were significant, it still indicates that tigers are indeed able to distinguish between the different categories of scent marks, both the intra- as well as the interspecific ones. These results are a tentative support for

Hypotheses 6, 7 and 8.

Indoor bioassays appear to be more rewarding to the experimenter than outdoor ones in that a higher proportion of the marks presented to the animals are responded to. Furthermore, a small outdoor enclosure results in a higher response rate than a large one. Thus bioassays should be performed in the night cages to obtain maximum efficiency.

When wild tigers pass a marking post they may react to the scent mark in varying intensities of *Scenting*, *Flehmen*, *Licking* and *Overmarking* (Schaller 1967, McDougal 1977). For the captive tigers *Scenting* and *Flehmen* were by far the most common types of reaction when encountering a foreign scent mark. These two response types were investigated in more detail. Generally the intact male in KBH showed higher levels of *Scenting* and *Flehmen* towards most of the experimental mark types than either females, castrated males or cubs (castrates). He showed more interest in tiger male marks (both *Scenting* and *Flehmen*) and tiger female oestrous marks (*Flehmen*) than in tiger female marks, and indeed these two mark types are the ones which should be most interesting to a male tiger from an evolutionary point of view. Male tigers compete for territories and a pool of younger nomadic males are roaming around on the lookout for an unoccupied area in which to establish themselves. Therefore territorial holders should recognise the presence of intruders by their scent marks and evict them if they meet, whereas intruders should recognise areas which are occupied and spend a minimum of time in them unless they wish to challenge the territorial holder. As male and female tigers have no regular social interactions male tigers should recognise the presence of an oestrous female in his territory by her scent marks and in this way to be able to find her and mate her while her oestrous period lasts. In this particular case vocal communication is also important as oestrous females are known to vocalise regularly.

The female tigers showed no particular interest in any of the scent mark categories over the other. As females are individual territorial holders like the males one might have assumed that marks from foreign female tigers would have been of particular interest to the females as they would have been possible competitors for the territories, but this did not appear to be the case.

The castrated males, contrary to the intact male, showed no interest in other male tiger marks and this indicates that castration also have an effect on the olfactory response of the animals. In contrast they showed considerable interest in marks from lions, and this strengthens the point that the lack of response to male tiger marks was a true negative response rather than a general unresponsiveness to scent marks due to the castration.

The male tiger in KBH, Sym, was tested for his ability to discriminate individual scents. He showed a clear pattern of habituation when presented with three identical scent

marks, but no habituation response to a fourth mark of different origin presented alongside the other three. This is considered a strong indication that tigers are able to distinguish between individuals by their scent and it thus provides support for Hypothesis 4.

4.1.4 Chemical analysis.

This section presents the results of the chemical analysis of tiger urine samples which were carried out in order to collect data which could be used in the discussion of the validity of Hypothesis 5, i.e. that differences exist in the chemical composition of scent samples (page 20).

Four new individuals are introduced into the analyses at this point. Urine samples from two additional adult intact males and two adult females were supplied from two other Zoological Gardens in Denmark. The adult males were Ramoz from Odense and Rubi from Aalborg, and the latter zoo also provided samples from the two females Felina and Vela.

Results.

On the basis of the identification process a list of 80 different compounds was produced, each of them being found in one or more of the 19 samples analysed. Of these 80 compounds 23 were also found in the accompanying control samples of laboratory air. Therefore only 57 compounds can be said with certainty to originate from the tiger urine, whereas the other 23 compounds may have had a different origin. All 80 compounds are listed in **Table 4.1.1** showing their names and retention times as well as in which individual(s) they were found. In Table 4.1 are also included the number of samples analysed for each individual and the total number of compounds identified for these individuals excluding those compounds which were also found in the corresponding control samples of laboratory air. These latter compounds have been excluded in the analyses which follows.

Of the compounds identified numbers 2, 4, 10, 17, 23, 38, 39, 53, 59, 65, 68 and 72 were exclusive to samples from intact male tigers, though they were not all found in every intact male tiger sample. Compounds numbers 22, 24, 55, 64, 74 and 79 were found in one or more female tiger samples only. Compounds numbers 18, 25, 29, 37, 75 and 80 were found in samples from castrated male tiger cubs only. The rest were found in one or

more samples from both sexes.

Based on the data in Table 4.1 an Average Linkage Cluster analysis was performed to investigate the underlying trends in the relatedness of compound composition for the different tigers. The dendrogram in **Figure 4.1.17** show the result of the analysis. There are no obvious separations between males and females, or between the intact males and the castrates.

Figure 4.1.18 shows the percentage overlap in compound composition between the sex groups including the castrated male cubs. As with the lion analyses the percentage overlap between two individuals or groups is defined as the percentage of the total number of compounds which are common to both, i.e. if a male has 20 compounds in his urine, and a female has 15, and they share 10 compounds, then the overlap from the males point of view will be 50% whereas it will be 67% from the females. No significant differences were found in the percentage overlap between any of the sex groups.

In **Figure 4.1.19** the mean overlap in compound composition within each of the individuals and the overlap of each individual with the other individuals of the same sex are compared. Only Sym has a "within overlap" which is significantly higher than his "between overlap" ($t=2.20$, $DF=14$, $P=0.045$), the rest of the tigers all have a higher "within overlap" than "between overlap" but not significantly so.

A number of the compounds listed in Table 4.1 was found to be present in almost all individuals of both sexes as well as in the samples from the castrated cubs. These were compound numbers 1, 12, 15, 20, 30, 42, 61 and 69, excluding the compounds which were also found in control samples. The absolute concentration (TIC) of each of these compounds was measured as the areas underneath each of the respective peaks on the chromatograms. The mean value for each of the sex groups in absolute concentration for each of these eight compounds are shown in **Figure 4.1.20**. The only significant difference which was found between the sex groups was for compound 30 (5-hydroxy-4-methyl-6-hepten-3-one) of which the castrated cubs had a significantly higher value than the intact males ($t=41.15$, $DF=2$, $P<0.001$).

The mean percentage concentration of the eight compounds for males, castrated cubs and females are shown in **Figure 4.1.21**. Only one significant difference was found between the sex groups, namely the castrated cubs' higher percentage concentration of compound 30 than the intact males ($t=6.08$, $DF=2$, $P=0.026$).

Discussion.

Tigers are much more solitary animals than lions and rarely have prolonged interactions with other individuals, except when a male is with an oestrous female or when a female is with her dependent cubs. Therefore the use of very highly volatile compounds for instantaneous short-term communication should be less important for the tiger than for the lion. Instead medium and, especially, long-term communication should play the major role. Compounds of high, medium and low volatility were found in tiger urine marks, so at a first glance there does not appear to be any special adaptation for long-term communication in the tiger. A tiger could also extend the life of its scent mark by producing a higher concentration of lipids in its urine, which would then be deposited together with the scent mark. This does in fact seem to be the case. As mentioned previously it has been established that tiger urine contains a high proportion of whitish lipids which are clearly visible to the human eye. In contrast lipids are not directly visible in lion urine. The lipid portion of tiger scent marks have a very long life. The black tar-like mass will last for many months even under unfavourable weather conditions.

A wide range of compounds was identified in the samples analysed in this study. The discussion below is divided into two sections, one on the amines found in the samples and the other on the non-amines found. This division is adopted for the sake of comparison with previously published papers and it does not necessarily signify that amines are more important in communication than the other classes of compounds.

Amines.

The first amine identified in the marking fluid of a tiger was 2-phenylethylamine (Brahmachary and Dutta 1979, 1981, 1987). This finding was later disputed by Banks *et al.* (1992) who detected no trace of the amine in samples from two tigresses. In this study no trace was found of 2-phenylethylamine despite samples from nine different animals being analyzed, so its presence in tiger urine must be seriously questioned. Brahmachary and Dutta (1981) and Banks *et al.* (1992) both found putrescine (1,4-butanediamine) in tiger marking fluid but this compound was not identified in the present study.

Trimethylamine was also found by Banks *et al.* (1992) when they analyzed marking fluid from a Bengal and a Sumatran tiger, indeed it was the most abundant of the seven amines they identified. This amine was also identified in the present study but none of the other amines found by Banks *et al.* was seen in this study. Conceivably this could be due to a difference between the sub-species of tigers studied, but as Banks *et al.* (1992)

analyzed samples from both a Bengal and a Sumatran tiger, and found the same range of amines this does not seem very likely. In my study trimethylamine was found in three out of four female tiger samples, and in two out of three intact male samples plus one sample from a castrated male cub.

3-methyl-1-butylamine has not been reported in tigers by previous workers. This is surprising as it was found in samples from all three intact male tigers, and three out of four female tigers in the present study. It was found in only one of the castrated animals and then in only one sample. Likewise n-ethyl-1-propylamine has not previously been identified for tigers and in this study it was present only once in a sample from one of the castrated male cubs. The two unknown amines (No 22 and 55) were identified twice altogether, compound 22 in a sample from a female, and compound 55 was seen in one sample from another female.

Based on this evidence it seems that amines are regularly found in tiger urine. Most of the amines identified are relatively small and volatile compounds, so they might serve a purpose in short term communication but this is still purely speculative.

Non-amine compounds.

A study of the non-amine fraction of tiger urine was made in 1987 by Brahmachary and co-workers. They found evidence of hydrocarbons with a carbon chain length from 10 to 16, and speculated that shorter chain compounds might also be present, but this aspect was not investigated. There was evidence of a cyclohexane-like compound. A number of free fatty acids were present in the samples, but a positive identification of them was not obtained (Brahmachary *et al.* 1987). In a later paper they list 14 free fatty acids that were identified from a tiger urine sample (Poddar-Sarkar *et al.* 1991). The lipid part of the urine has also been analysed and it was found to comprise cholesterol ester, wax ester, triglyceride, free fatty acids, diglyceride, monoglyceride, free sterol and phospholipid (Poddar-Sarkar 1996).

In the present study the most common non-amines present were three ketones (No 12: 2-butanone, no. 61: 2-heptanone and no. 69: 2-undecanone). These compounds were found in the majority of the samples from all individuals. A number of other non-amines occurred in various samples but more infrequently than the three listed above. No free fatty acids were identified but this was probably due to the general unsuitability of the method used to detect this type of compound.

Can tigers use scent marks deposited by foreign animals to identify their individual

identity, sex or species and if so how do they do it?

In the scent presentation experiments described in the previous section an indication was found that tigers react differently to scent marks from different individuals. This difference in reaction must be based on an ability to distinguish between the scent marks on the basis of their chemical composition. Have the results from the chemical analyses presented above shown any major differences in the chemical composition of scent marks between individuals or sex groups?

Three of the 57 compounds, namely 2-butanone, 2-heptanone and 2-undecanone, identified from the tiger urine samples were present in all of the intact individuals, so it is possible that one or more of these might carry a message of species identity. However, before the composition of other felid species cohabitating with tigers are analysed it is difficult to draw any firm conclusions on this matter.

No compound was found in all individuals of one sex and not in the other, leading to the conclusion that none of the compounds identified in this study acts as a universal sex-identifier on its own. However, some compounds were found only in male samples, though not in all male samples, and similarly some compounds were identified only from female samples. These compounds might belong to a pool of "male" and "female" compounds respectively, so that when an animal encounters any of these compounds it is able to identify whether the scent mark was deposited by a male or female tiger.

On the quantitative level only one difference in compound concentration between males and castrates was found. Both with regards to absolute and relative concentration, castrated cubs had a higher level of compound 30 (5-hydroxy-4-methyl-6-hepten-3-one) than intact males. This implies a possible androgen influence on the urine composition of tigers, and this phenomenon has also been seen in other species such as mouse and wolf (Schwende *et al.* 1986, Raymer *et al.* 1986). No quantitative differences were found between male and female scent samples in the present study, but once a complete chemical description of tiger urine is obtained such differences are likely to be found as indicated by the scent presentation experiments.

The high variability in compound composition within the individual also makes it difficult to draw conclusions on the basis of discrimination between individual scents in the tiger. However, no two individuals had a 100% overlap in compound composition so this may form at least part of the foundation for individual discrimination. A significantly higher "within overlap" than "between overlap" was found for only one of the tigers even though the "within overlap" was higher for all the tigers. This is an indication that qualitative factors are also involved in the discrimination of scent samples.

These results provide a certain degree of support for Hypothesis 5, in that both qualitative and quantitative differences were found between individuals and sex groups even though the sample size in the tiger section is smaller than what would have been desirable.

4.1.5 Summary.

The following main findings with regards to scent marking and chemical composition of urine were presented for tigers in this chapter. The number of individuals in the different sex groups are somewhat low, and especially for intact males where only one individual was available in the behavioural part, and therefore the results should be treated with caution.

The scent marking repertoire of all individual tigers is described. No significant difference in the percentage of *Spraymarking* was found between either of the sex groups. The intact male had a significantly higher rate of *Spraymarking* than the castrates as did the female tigers. No significant difference was found between the rates of the intact male and the females. A male which was castrated just before the beginning of the fieldwork showed a declining rate of *Spraymarking* over this period.

The intact male tiger showed more interest in marks from other male tigers and females in oestrous than the other categories of scent marks. The castrated males had a reduced interest in other male tiger marks.

Tigers were shown to be able to discriminate between two scent marks of different origin.

Urine from tigers was found to contain 57 different chemical compounds. A number of compounds was present in samples from only the intact males, the females or the castrated male cubs.

No significant difference was found between the mean overlap of male samples with those of female samples, or *vice versa*. All tigers overlapped more within their own samples than between theirs and other samples from same sex individuals, but only one significantly so.

No significant difference was found in either the absolute or relative concentration between the intact male and the females for common compounds. The castrated male cubs had a significantly higher absolute and relative concentration of 5-hydroxy-4-methyl-6-hepten-3-one than the intact male.

4.2 THE LEOPARD: *Panthera pardus*.

The leopard is also a solitary *Panthera* species in which each of the sexes inhabit territories from which they exclude animals of the same sex. This imposes a restriction on the number of leopards that can be housed in the same enclosure and numbers in captivity are therefore low compared to the lion. In the zoos included in the behavioural part of this study only one had a group of leopards. This was KBH in which one male (Sorte) and one female (Plet) leopard were present at the beginning of the fieldwork period. They were housed in the same night cages. Later in the period the female gave birth to a litter of two and these young cubs (Blacky and Spot) were also included in the observations. After a period of some weeks just after the birth where the female and cubs were housed separately all four leopards were again housed in the same night cages.

4.2.1 Activity.

The data on activity are used in the evaluation of the effect of the captive environment on the leopards' basic behaviours, and to assess possible influences on the scent marking behaviour.

Results.

The main activity for both the adult leopards and the cubs was *Lying* (56% \pm 9.5). This was followed by *Moving* (24.3% \pm 10.1), *Other* (8.7% \pm 5.5), *Standing* (5.7% \pm 2.3), and *Sitting* (5.3% \pm 2.1) (Figure 4.2.1). An overall significant difference in activity between the leopard sex/age groups was found ($\chi^2=155.9$, DF=8, $P<0.001$). Figure 4.2.2 shows the contribution of the individual cells in the Chi-square test to the overall value. The figure includes the direction of the deviation i.e. whether a particular group showed relatively more or less of each activity compared to the other groups. The main contributor to the overall value was the high level of female *Moving* and the high level of cubs doing *Other* activities. Other values that differ between the sex/age groups were the low level of male and cub *Moving*, the low level of cub *Standing* and *Sitting*, the low level of female *Lying*, and the low levels of male and female doing *Other* activities.

Discussion.

The leopard is generally described as a nocturnal hunter (Schaller 1967, 1972) but several accounts of activity during the daytime were also found (Myers 1976; Norton and Henley 1987; Bailey 1993). A detailed activity budget for the wild leopard could not be found as the studies reported in the literature have all been made by radio- or spoor-tracking. Bailey provides some data on visual observations of leopards. He reported that of 81 encounters by day the leopard was *Moving* in 54%, *Lying/Resting* in 44% and doing *Other* behaviours (given as "Courting") in 2% of records. At night encounters *Moving* made up 90%, *Other* 7% and *Lying/Resting* only 3% of the records. As Bailey's observations were not systematic there is likely to be a heavy bias towards moving animals, especially at night. It is difficult therefore to put my own activity data into context and to say whether the higher activity of the female is peculiar to these particular animals or whether it might be a species specific trend. Exner (1995) provide some activity data for captive leopards in German zoos. She found that they spent 34% of the time *Moving*, 7% *Sitting* and 51% *Lying*. For the remaining 8% of the time the leopards were out of sight. These figures compare well with those found for the KBH leopards, but Exner did not divide her data up into sex/age groups which makes a more detailed comparison difficult.

It therefore appear that the activity of the KBH leopards fall well within what is seen for other captive populations and also for wild leopards. This reduces the possibility that abnormal levels or types of activity have influenced the scent marking results presented later in this chapter.

4.2.2 Social behaviours.

The results on leopard social behaviour shown in this section are also part of the baseline data used in the evaluation the effects, if any, which abnormal behaviour might have on the scent marking of the animals.

Results.

The first analysis employed in the investigation of leopard social behaviour is a qualitative one. The directions of social interactions within the leopard group are shown in **Figure 4.2.3**. The following trends were evident.

Headrubbing: *Headrubbing* was mostly seen between the adults.

Bodyrubbing: This behaviour was performed only among the adults.

Allogrooming: *Allogrooming* was directed by all members of the group towards all other members of the group except from the cub Spot towards his brother Blacky.

Playing: *Playing* was observed bilaterally throughout the group.

Investigating: *Investigating* was seen between the adults and between the adult male Sorte and the cub Spot.

Mild Aggression: *Mild Aggression* was observed between the adults, and with the adults as actors targeting the cubs, but never with cubs targeting adults. It was also seen between the cubs.

Serious Aggression: *Serious Aggression* was mainly seen between the adults, but Sorte and Blacky were also actors with Spot as the target.

Mating: Only the adult male Sorte was observed to display mating behaviour with the adult female Plet always as target, i.e. the female did not actively encourage the male to mate with her.

Following the qualitative study of leopard social behaviour, a quantitative analysis of the six most frequently occurring social behaviours was made. The results of the percentage distribution of these six behaviours within the group are shown in **Figure 4.2.4**. It is evident that there is a big difference between adults and cubs, and minor differences between the two adults. The overall difference between the three sex/age groups was highly significant ($\chi^2=441.6$, $DF=10$, $P<0.001$). In **Figure 4.2.5** the overall difference is broken down into the contribution of each of the cells in the Chi-square test. The major contributions come from the low level of *Mild Aggression* and high level of *Playing* for the cubs, the high level of *Investigating* by the male, and the high level of *Allogroom* and *Mild Aggression* as well as the low level of *Playing* by the female.

In order to analyse the patterns of *Non-Aggressive* and *Aggressive* interactions between sex/age groups, the *Non-Aggressive* behavioural categories and the *Aggressive* behavioural categories were each pooled separately. The *Non-Aggressive* behaviours were *Headrubbing*, *Bodyrubbing*, *Allogrooming*, *Playing* and *Investigating*, and the *Aggressive* categories were *Mild Aggression* and *Serious Aggression*. The results of this analysis are

shown in **Figure 4.2.6**. The male received different levels of *Non-Aggressive* and *Aggressive* behaviours from the female and cubs with the female pattern being dominated by *Aggressive* and the cubs pattern by *Non-Aggressive* behaviours. The difference between the two patterns was significantly different ($\chi^2=68.8$, $DF=1$, $P<0.001$). The female received equal amounts of *Non-Aggressive* and *Aggressive* behaviours from the male and only *Non-Aggressive* behaviours from the cubs. Again the difference between the two patterns was significant ($\chi^2=39.2$, $DF=1$, $P<0.001$). Cubs received equal amounts of the two categories from the male, and more *Non-Aggressive* from the female. Between the two cubs *Non-Aggressive* behaviours were very dominant. The *Non-Aggressive* and *Aggressive* patterns shown by the three sex/age groups towards the cubs were significantly different ($\chi^2=97.4$, $DF=2$, $P<0.001$).

Discussion.

Leopards are considered to be highly asocial (Schaller 1967, 1972; Myers 1976; Bailey 1993, Jenny 1996). The only regular social interactions are likely to be those between a mother and her cubs. Therefore the social environment of the zoo where a male is kept permanently with a female and her offspring is very unlike the normal social environment of the leopard. One aspect which definitely varies is the frequency of interaction, which will be much higher in the captive environment. Social interactions between wild leopards are very rarely observed and there are no quantitative and very few qualitative data from the wild with which to compare my own data. Exner (1995) found that 24% of interactions between her zoo animals were *Headrubbing* or *Allogrooming* ("Kopfreiben" or "Lecken"), 36% were *Playing* ("Spielen"), 32% were *Mild Aggression* ("Drohen") and 7% were *Serious Aggression* ("Angreifen"). Again her data are not divided into sex/age groups. These numbers compare well with the interaction percentages observed for the adult male and female in KBH. The male's *Investigating* was directed towards the female and is probably part of normal oestrous screening behaviour. That the cubs had relatively high levels of *Playing* behaviour and low levels of aggression is hardly surprising as they are the weakest of the social groups. They could of course show aggressive behaviour among themselves but this was rarely the case. The cubs interacted primarily with each other and showed equal proportions of interactions with the adult male and female.

4.2.3 Scent marking.

This section presents results on the scent marking behaviour of the leopard group. These data were recorded to test the validity of Hypothesis 1 (page 19) for leopards by looking for differences in scent marking between the male and the female. With only one individual in each of the sex groups the results will be preliminary, but they can still give an indication of whether the Hypothesis holds.

The female leopard Plet ceased to scent mark altogether after giving birth to the cubs and she had not resumed scent marking when the fieldwork ended about 6 months later. The scent marking data on the female leopard, therefore, are restricted to the period before the birth of the cubs.

Scent presentation experiments were performed on the leopards to test Hypotheses 6, 7 and 8, i.e. their ability to distinguish between different categories of scent marks.

In all experiments distilled water was used as a control mark. No reactions were ever seen towards the water marks and consequently they have been left out of the following analysis.

Defaecation was observed infrequently and there was no indication of the faeces being deposited in any special way. Faeces were never associated with scrapes. Therefore *Defaecation* has been left out of the following analyses.

Results.

Scent marking repertoire.

The first results on the scent marking behaviour of the leopards are for the percentage distribution of the different marking types for the two adults Sorte and Plet (**Figure 4.2.7**). There were quite pronounced differences between the male and the female. Sorte had equal amounts of *Spraymarking* and *Scrape/urination* in his repertoire whereas Plet's was dominated by *Urination* and *Clawing*.

Scent marking rates.

The rates of marking for Sorte and Plet are shown in **Figure 4.2.8**. Sorte had high rates of *Spraymarking* and *Scrape/urination*. All marking rates for Plet were much lower than for Sorte.

Investigation of natural scent marks.

Only two reactions to natural marks were observed. These were of the male and female investigating a *Scrape/urination* made by the male. Both reactions consisted of *Scenting* and *Flehmen*. No instances of *Licking* or *Overmarking* of natural scent marks were observed.

Presentation and reaction to experimental scent marks.

In **Figure 4.2.9** the response preference of the leopards to different types of experimental marks is shown by use of a Chi-square test with the individual cells indicating whether a given mark type was reacted to more (positive values) or less (negative values) than expected relative to the other mark types. For the male it is clear that Female leopard marks were reacted to less than expected whereas Lion marks were reacted to more than expected. Neither the female nor the cubs had any major deviance from the expected values.

When investigating scent marks leopards exhibit four different types of behaviour *Scenting*, *Flehmen*, *Licking* and *Overmarking*. The last two types were rarely seen in KBH but the first two were quite common. These two were investigated in more detail when looking at the leopards' responses to the different types of experimental marks presented to them. The frequencies of *Scenting* towards the different types of experimental marks for the leopard sex/age groups in KBH Outdoor and Indoor experimental series are shown in **Figure 4.2.10**.

Male leopard: No significant difference was found in the male's *Scenting* frequency towards the different scent mark types in either KBH Outdoor or Indoor series.

Female leopard: No significant difference was found for the female either.

Cub leopard: No significant difference was found for cub *Scenting* behaviour.

Comparison between sex/age groups:

KBH Outdoor: The male leopard showed significantly more *Scenting* towards both Lion and Tiger marks than did the cubs ($P=0.002$ and $P<0.001$ respectively). The female showed significantly more *Scenting* than the cubs towards Tiger marks ($P=0.008$).

KBH Indoor: The female leopard had higher *Scenting* frequencies than the cubs towards Leopard male marks ($P=0.003$) and Lion marks ($P=0.041$).

The frequency of *Flehmen* towards the different types of experimental scent marks are shown in **Figure 4.2.11**. As seen for *Scenting* there were no significant differences within any of the three sex/age groups in their *Flehmen* frequencies towards the experimental scent mark types.

Comparison between sex/age groups:

KBH Outdoor: The male leopard showed significantly more *Flehmen* towards both Lion and Tiger marks than did the cubs ($P=0.015$ and $P=0.024$ respectively).

KBH Indoor: No significant differences were found between the sex/age groups in this series.

Discussion.

The scent marking behaviour of the leopard has been recorded by several researchers. Among the earliest accounts are those of Schaller (1972) and Bertram (1978) who made observations on leopards during their several years of studying lions in the Serengeti region of Eastern Africa. They noted that wild leopards of both sexes used *Spraymarking* and *Scrape/urination* as part of their communication system. They both found that *Spraymarking* was more common than *Scrape/urination*. Rabinowitz (1989) found a seasonal variation in the number of scrapes encountered during his study in Thailand with the majority occurring during the rainy season. Bailey (1993) observed leopards in Kruger National Park in South Africa and found that both sexes used *Spraymarking*, and he also noted the occurrence of *Scrape/urination*. He did not observe leopards clawing trees. Bothma and le Riche (1995) did find evidence that leopards in the Kalahari use clawing of and rubbing against trees and branches alongside the other types of marking. Jenny (1996) studying the leopards of Taï National Park, Ivory Coast, found that the density of *Scrapes* was higher in an overlap zone between two female territories than in other parts of their range, and he also found evidence of scratching of trees. No observations have been reported to indicate that faeces are used in any special fashion by leopards. This was not observed in the present study either, so it appear that faeces do not play an important role in the communication system of the leopard. None of the above studies provides reliable

quantitative data for leopard scent marking. It is therefore difficult to discuss the scent marking observed in KBH in a broader perspective.

No other reports of *Panthera* females ceasing completely to scent-mark after giving birth have been found. It is therefore impossible to say whether it is peculiar to this specific female or whether it might be a more general phenomenon. However, Seidensticker *et al.* (1973) reported that female Mountain lions (*Felis concolor*) with cubs did not perform scrapes, which is the main type of marking in this species. The rate of scent-marking by the leopard male was considerably higher than that of the female, but data on more individuals need to be included before any definite conclusions can be drawn on whether this is a general trend or not.

The reaction of leopards to scent marks is described as one of investigation, possibly followed by *Flehmen* and sometimes also counter marking (Bailey 1993). No quantitative data on these reaction types are available from the literature. Few general trends are evident from the results obtained in this study. The two adults showed significantly higher levels of *Scenting* and *Flehmen* towards many of the experimental mark types than the cubs, and this difference must be put down to age rather than any other influence. The male had a strong bias in *Scenting* towards Leopard male marks over Leopard female marks but the difference was not significant. As both males and females hold exclusive intra-sex territories (i.e. held exclusively by one sex), one would have expected to see more interest in marks from their own sex group than the other mark types i.e. because these would indicate the presence of competitors. As only one individual was tested for each sex group it is too early to rule out the existence of such a difference even though it was not very evident here. More tests on more individuals will have to be carried out before this point can be elucidated further.

4.2.4 Chemical analysis.

This section presents the results of the chemical analysis that were carried out on the leopard urine samples. These data were collected to provide a basis for the evaluation of Hypothesis 5 (page 20), i.e. that differences exist in the chemical composition of scent marks.

The table in this chapter show some of the samples as originating from individuals of unknown sex. This is because two individuals, the KBH male Sorte and the KBH female Plet, shared the cage from where the samples were collected, and it was not possible to tell which of the individuals had actually deposited the specific sample.

Results.

On the basis of the identification of the mass spectra from the leopard samples a list of 39 compounds found in these samples was compiled. Each of these compounds was found in one or more of the samples analysed. Of these 39 compounds 21 were also found in the corresponding control samples of laboratory air. Therefore only 18 compounds can be said to originate from the leopard urine with any certainty, whereas the other 21 compounds might have had different origins. The list is presented as **Table 4.2.1**.

If the "unknown sex" samples are disregarded compounds 1, 11 and 35 were found in male samples only, and compound 2 was found in a female sample only. However, all but compound 2 was also found in one or more of the "unknown sex" samples so their possible sex specificity should be treated with great caution.

Discussion.

Leopards are even more asocial than the tiger. In order for the chemical communication system of the leopard to be effective it should be similar to that of the tiger i.e. there should be no special need for very highly volatile compounds, suitable for short-term communication, but a greater need for compounds of medium and especially low volatility to enhance long-term communication. Leopard marks were the least complex of the three species analyzed and there did not appear to be any special bias towards low volatility of the constituents. In addition it has been shown that leopard urine does not contain lipids (Asa 1993).

A wide range of compounds was identified in the samples analysed in this study. As with lions and tigers the discussion below will be divided into two sections, one on the amines found in the samples and the other on the non-amines found. This division is adopted for the sake of comparison with previously published papers and it does not necessarily signify that amines are more important in communication than the other classes of compounds.

Amines.

Brahmachary and Dutta (1984) analysed the urine of two unweaned leopard cubs and

found phenylethylamine (PEA) to be the dominant amine. They also found putrescine. Since their finding of PEA in tigers has been questioned (Banks *et al.* 1992), the same doubt arises over their finding of PEA in leopards as its occurrence might be linked to their methodology. In this study the only amine found in leopard samples was trimethylamine, and even this occurred in only three samples, two from an adult male and one from an "unknown sex" animal. It is possible therefore that amines do not play a very important role in the chemocommunication of the leopard, but further analysis is needed to elucidate this point.

Non-Amines:

Only a few non-amines have been identified from the urine of the leopard. These are acetaldehyde, propionaldehyde and formaldehyde (Brahmachary and Dutta 1984, 1987). The present study did not confirm these findings, but this may well be due to differences in the method of analysis. A number of other non-amines was identified, the most common of which were 2-butanone (No 11), 1-phenyl-1-pentanone (or other $C_{11}H_{14}O$ isomer) (No 35) and nonanal+ methylbenzoate (No 36). Several other compounds were present but appeared less frequently in the samples.

Can leopards use scent marks deposited by foreign animals to identify their individual identity, sex or species and if so how do they do it?

None of the compounds identified from the leopard urine samples was present in all samples so it is unlikely that any of them carries a message of species identity. Because of the presence of the "unknown sex" category, it is difficult to assess the possible sex specificity of the identified compounds. Only compound 2 (pentane?) was found in one female sample alone. Whether this compound could be part of a pool of "female compounds" is impossible to say at this stage.

One must conclude that there are differences in compound composition between individual scent marks from leopards, but these are likely to be expanded as a more complete chemical profile of leopard urine is obtained. Whether there are quantitative differences between individual and sex groups also remains an open question. It is likely that specific differences in the chemical composition of leopard scent marks do exist, and that they will be found once a more extensive analysis is carried out. At present the data presented here are only indications of this.

4.2.5 Summary.

Only one group of leopards comprising one adult, one adult female and two cubs were included in the study. Therefore all results on leopards are tentative and preliminary findings which can only point in the direction of possibly interesting aspects. This is particularly true for the scent marking results, whereas the chemical data, which are based on the analysis of ten urine samples from leopards, are more well founded.

The male had higher percentages of *Spraymarking* and *Scrape/urination* in his marking repertoire than the female, and he also had higher marking rates for both mark types.

The adult leopards generally showed significantly more *Scenting* and *Flehmen* behaviour towards experimental marks than the cubs.

The leopard urine analysed contained altogether 18 different chemical compounds.

Figure 4.1.1: Activity of KBH and KNU tigers.
M: male, MC: castrated male, F: female, CC: castrated cub.

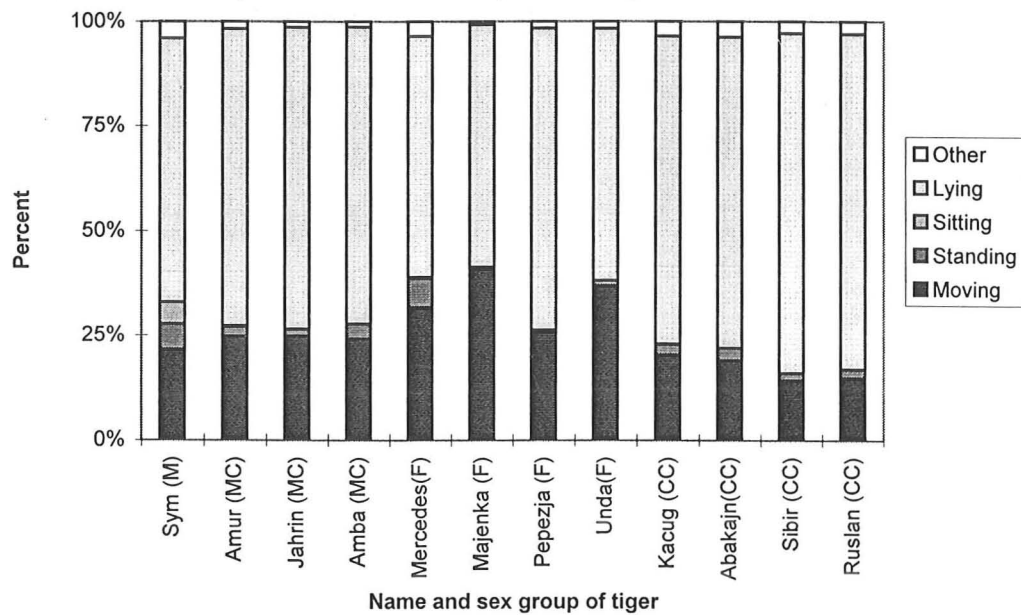
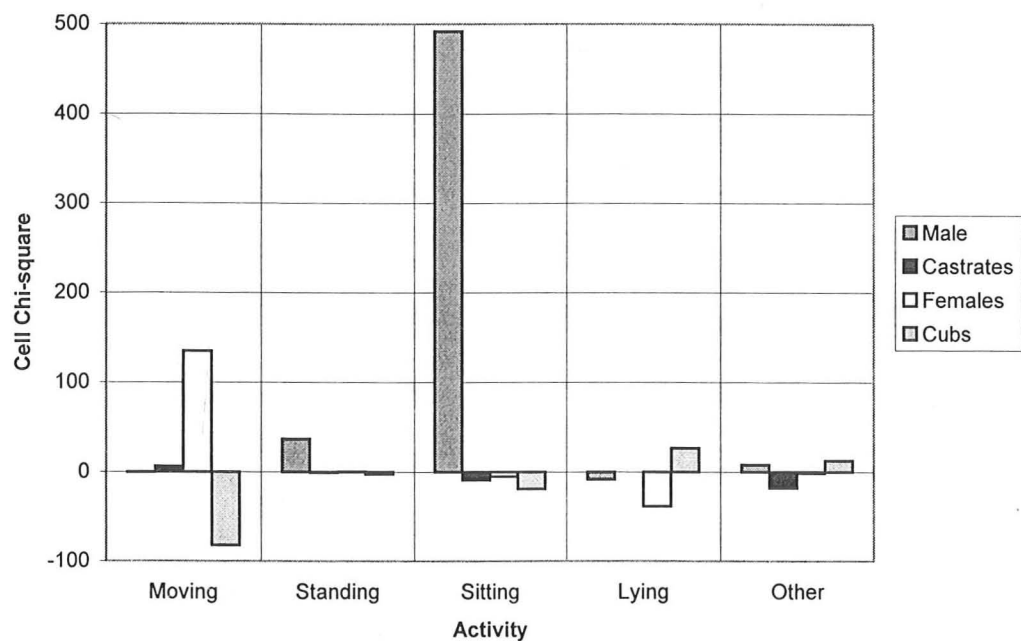


Figure 4.1.2: Activity differences between tiger sex/age groups.
Cell Chi-square values including direction of deviation.



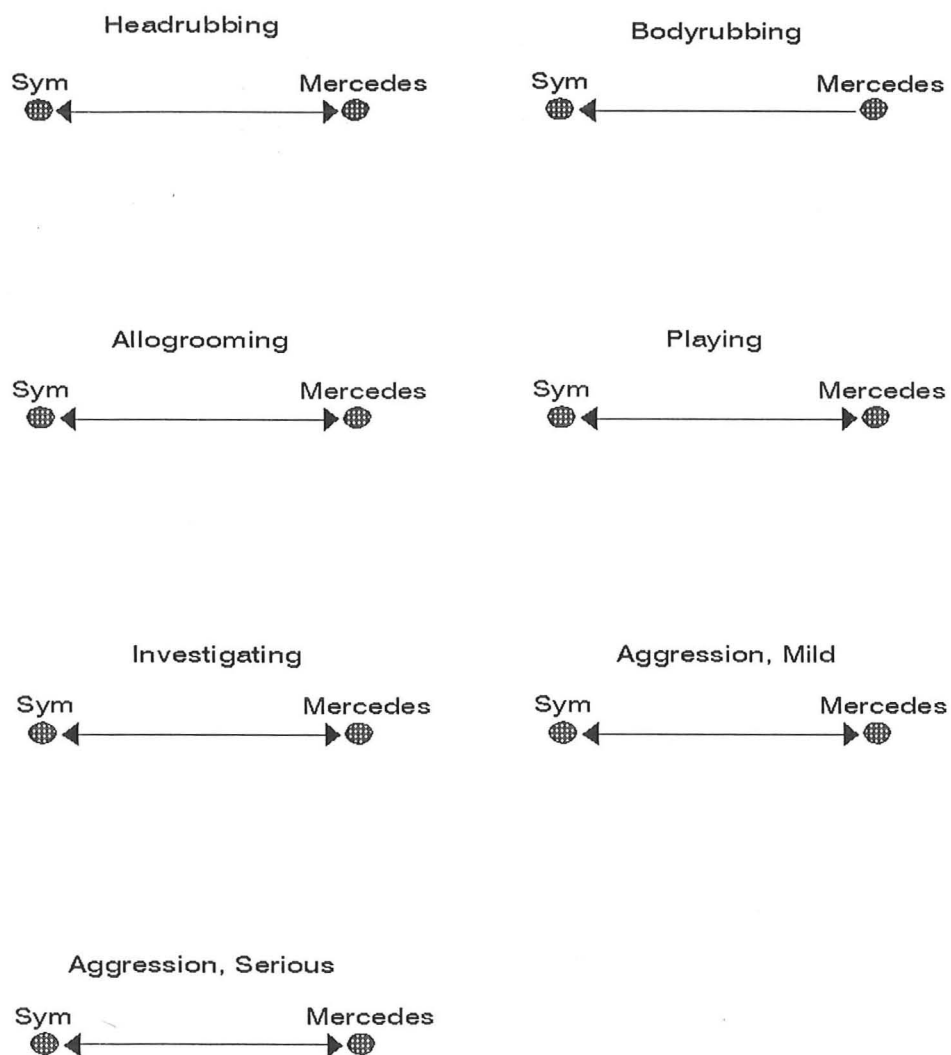


Figure 4.1.3: Qualitative social interactions between male (Sym) and female (Mercedes) tiger in KBH. Mating is not shown as it was not observed in these tigers. All relationships were mutual except for Bodyrubbing where only Mercedes was the Actor.

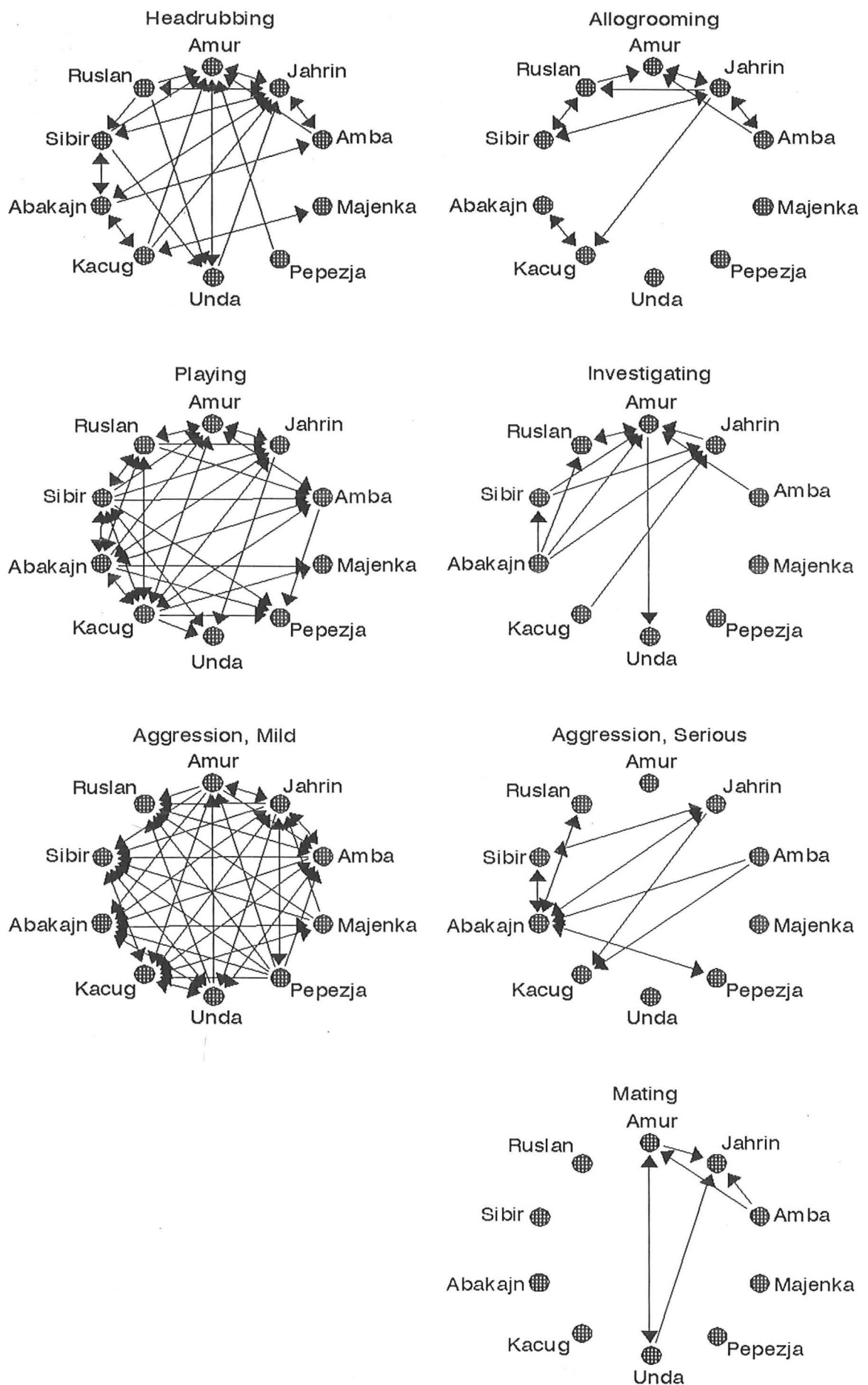


Figure 4.1.4: Qualitative social interactions between all individuals in the KNU tiger group. Bodyrubbing is not shown as it was not observed in these tigers. Arrows indicate direction of interaction from the Actor to the Target. MC: Amur, Jahrin, Amba; F: Majenka, Pepeza, Unda; CC: Kacug, Abakajn, Sibir, Ruslan.

Figure 4.1.5: Percentage distribution of the six most frequent social behaviours performed by the KBH and KNU tigers.
M: male, MC: castrated male, F: female, CC: castrated cub.

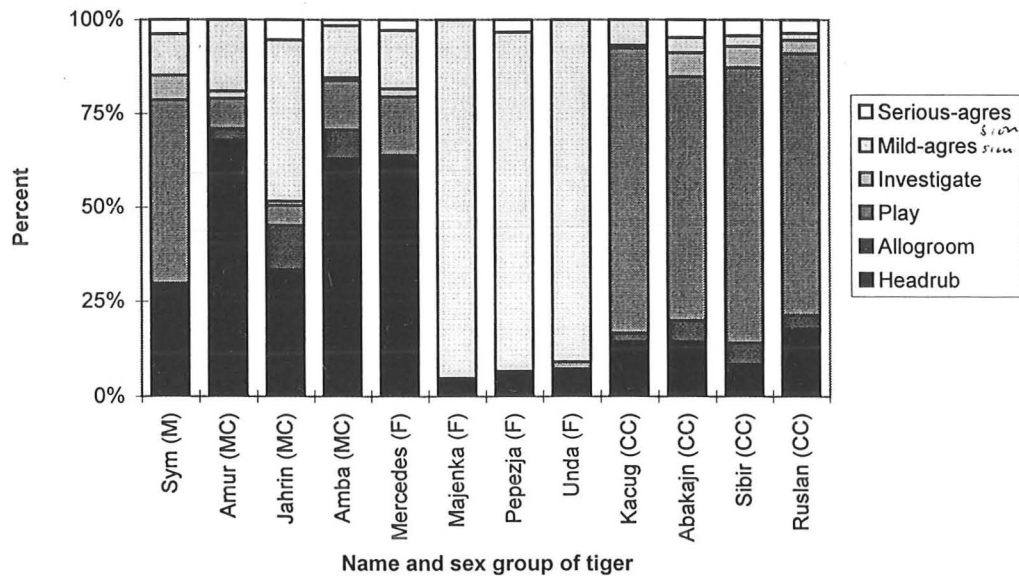


Figure 4.1.6: Social behaviour differences of tiger sex/age groups. Cell Chi-square values including direction of deviation.

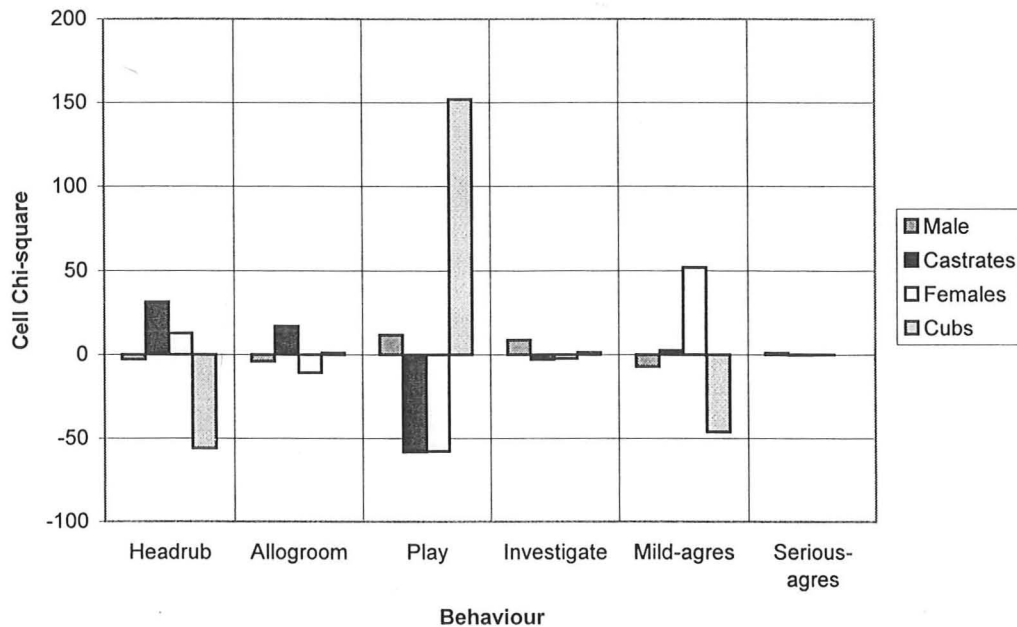


Figure 4.1.7: Number of Non-Aggressive and Aggressive social interactions between the two KBH tigers. Actor vs. Target individuals are indicated below the x-axis.

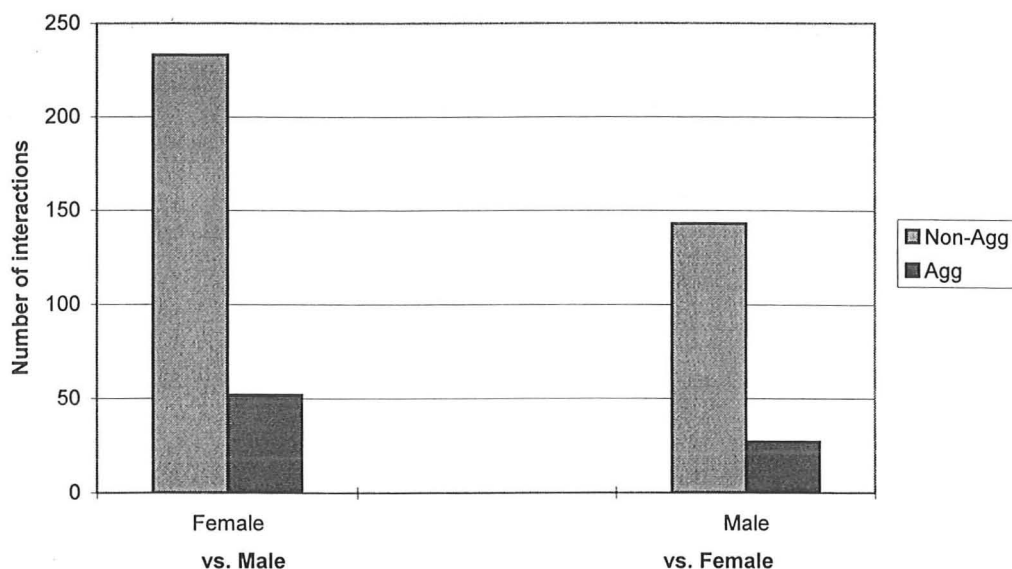


Figure 4.1.8: Number of Non-Aggressive and Aggressive social interactions between KNU tiger sex/age groups. Actor vs. Target individuals or groups are indicated below the x-axis. The three castrated males Amur, Jahrin and Amba are shown individually.

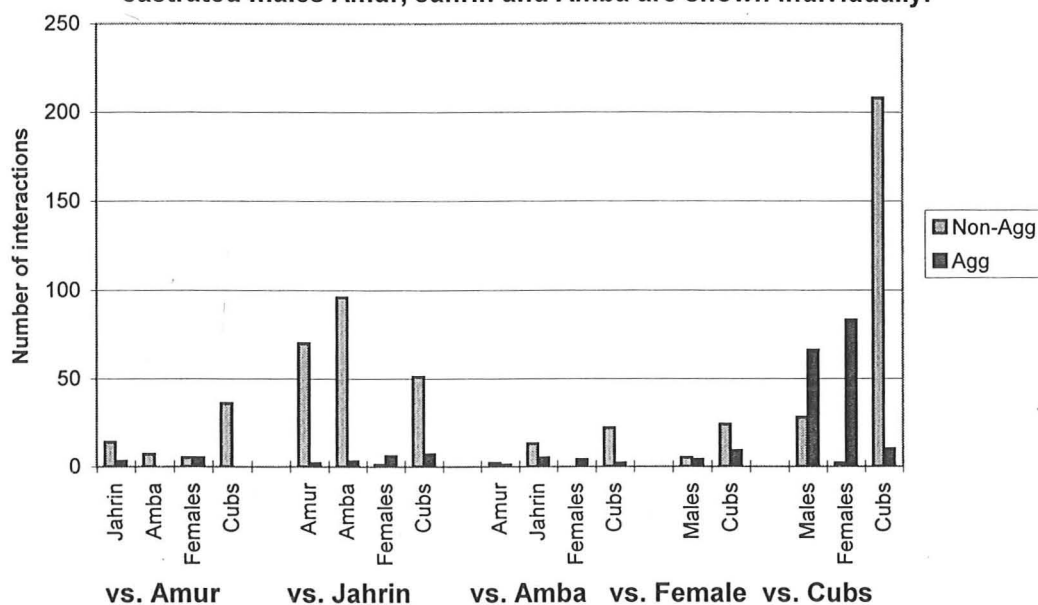


Figure 4.1.9: Percentage distribution of the different marking types for KBH and KNU tigers.

M: male, MC: castrated male, F: female, CC: castrated cub.

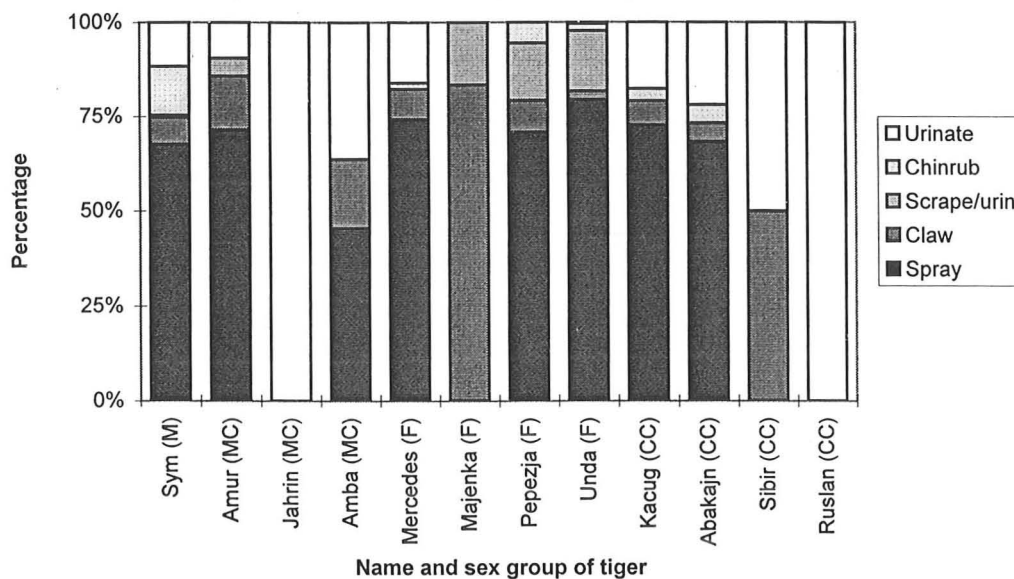


Figure 4.1.10: Marking rates for different mark types for KBH and KNU tigers.

M: male, MC: castrated male, F: female, CC: castrated cub.

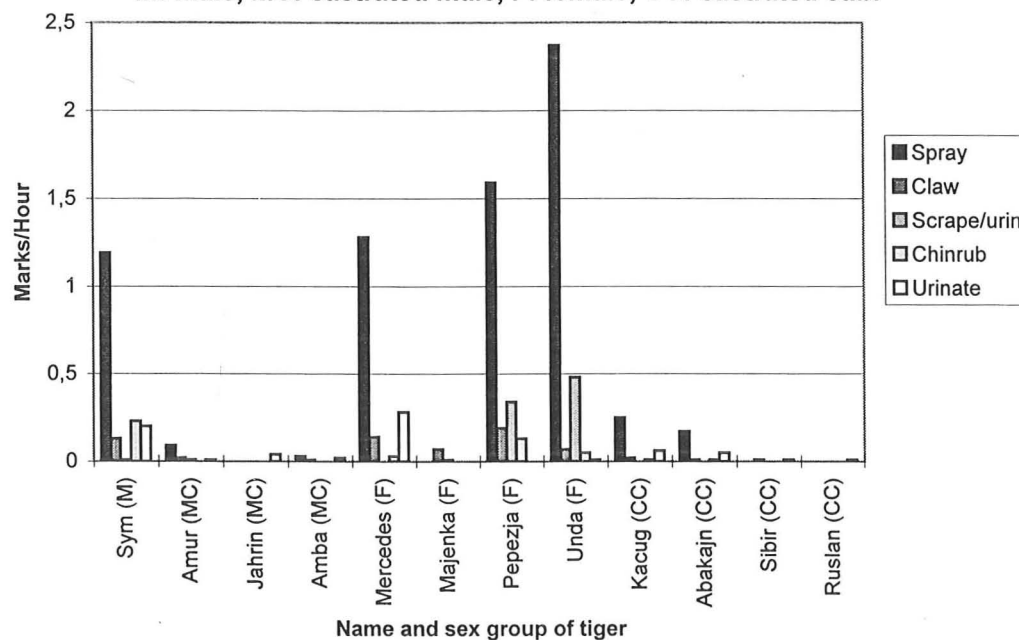


Figure 4.1.11: Patterns of Spraymarking and Urination for Kacug and Abakajn on days when they were together with their mother (Majenka) or a non-mother female (Unda).

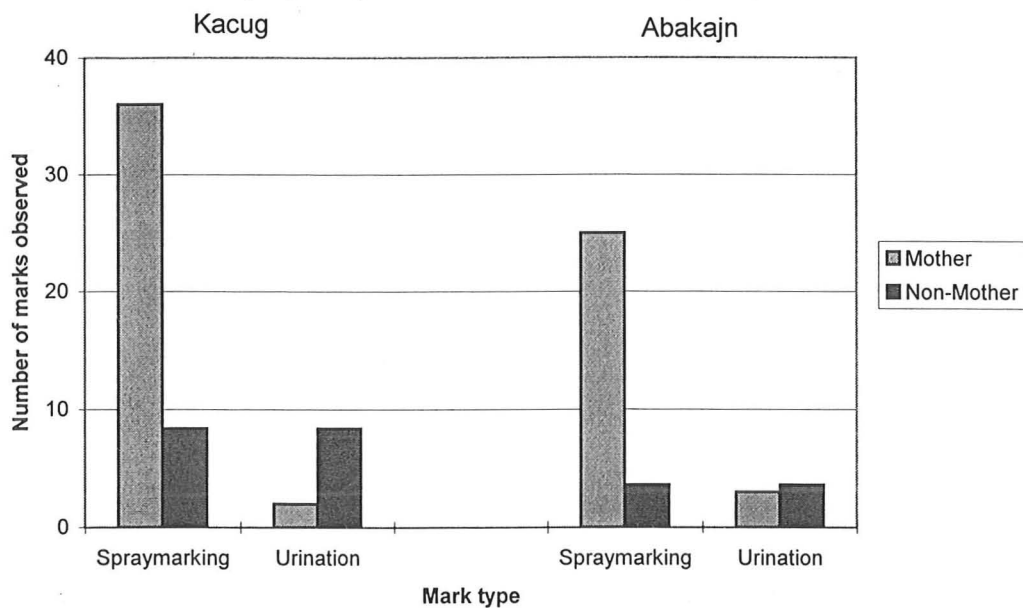


Figure 4.1.12: Change in Spraymarking rate in the KNU male Amur after his castration. No values are available for month 7, 9 and 11.

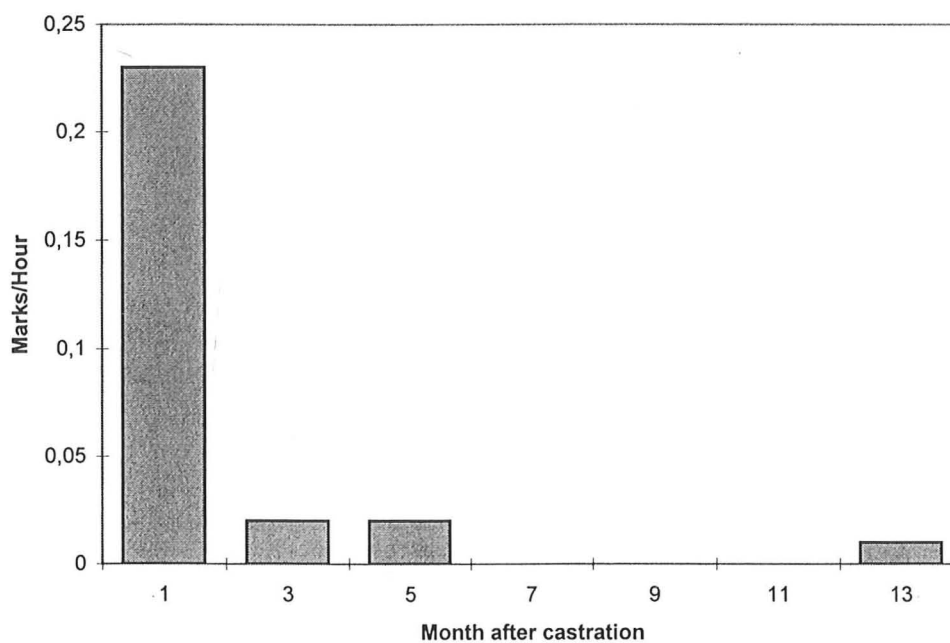


Figure 4.1.13: Combined data for KBH and KNU Indoor and Outdoor experimental scent presentations for tigers. Cell Chi-square values including direction of deviation.

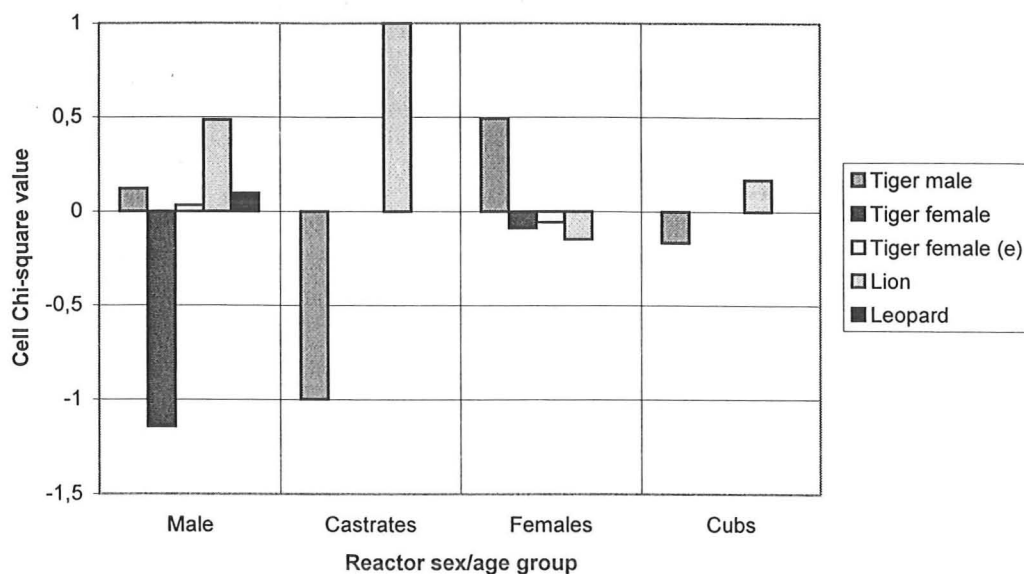
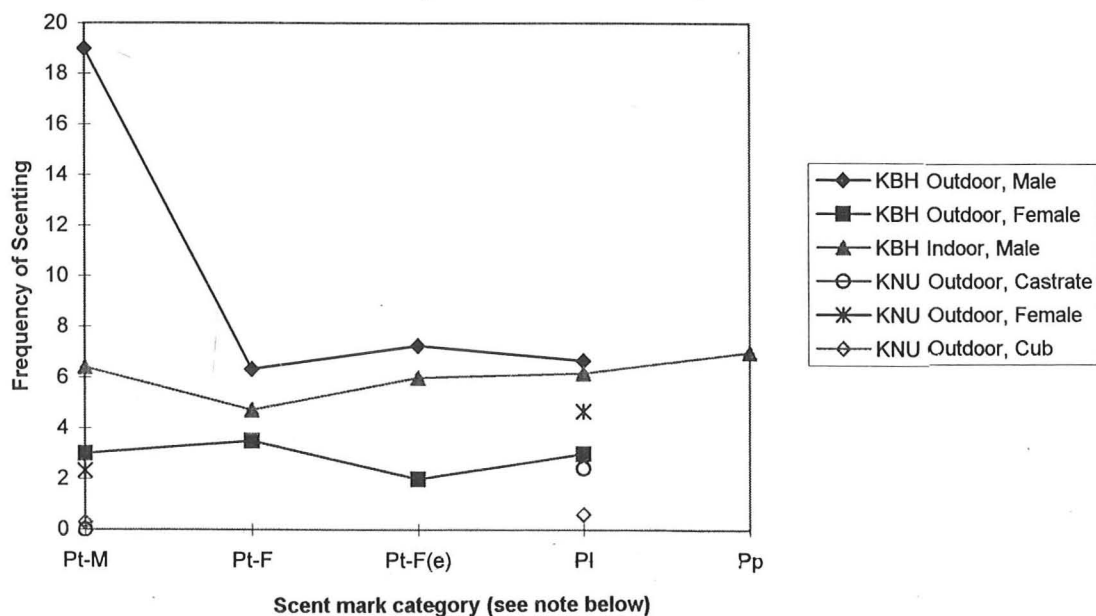


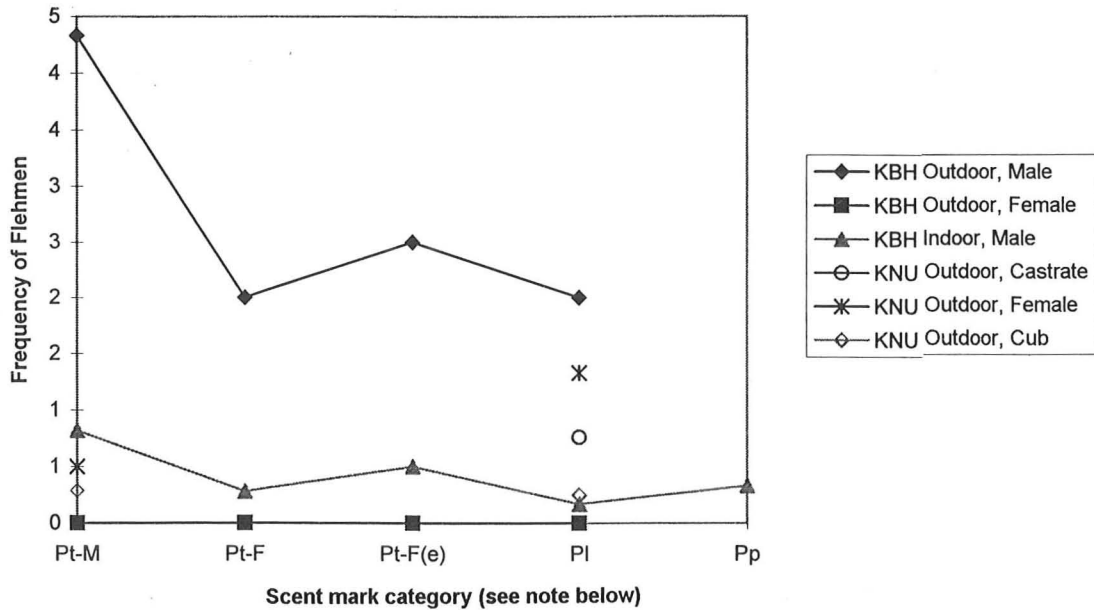
Figure 4.1.14: Frequency of Scentings (per 4 hrs presentations) for KBH and KNU tigers when investigating different experimental mark categories.



Note to Figures 4.1.14 and 4.1.15:

Scent mark categories: Pt-M: Tiger male mark, Pt-F: Tiger female mark, Pt-F(e): Tiger female estrous mark, PI: Lion mark, Pp: Leopard mark.

**Figure 4.1.15: Frequency of Flehmens (per 4 hrs presentations)
for KBH and KNU tigers when investigating different
experimental mark categories.**



Note to Figures 4.1.14 and 4.1.15:

Scent mark categories: Pt-M: Tiger male mark, Pt-F: Tiger female mark, Pt-F(e): Tiger female estrous mark, PI: Lion mark, Pp: Leopard mark.

Figure 4.1.16: Investigation of Indoor experimental marks by KBH male tiger. Like 1, 2 and 3 are identical marks, Odd is of different origin. Like 1 is the first mark encountered of its kind on a day, Like 2 the second and Like 3 the third.

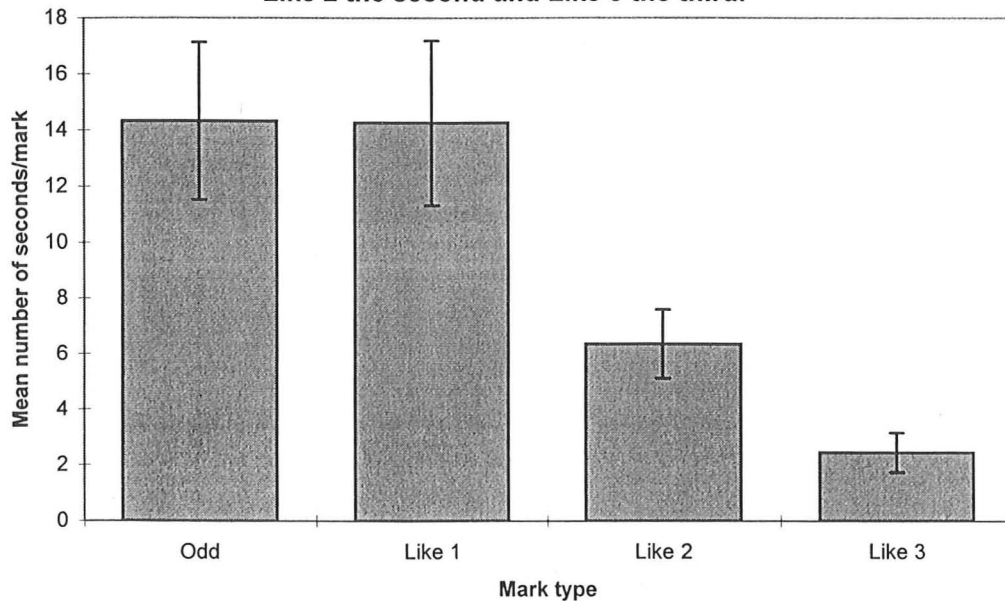


Table 4.1.1: Compounds present in the urine of male (Intact:n=3, Castrated male cubs:n=2) and female (n=4) tigers. "X" denote the presence of the compound in one or more of the samples analyzed. "0" indicates that the compound was present in both the sample and in a corresponding control (laboratory air) sample. At the bottom of the table the number of samples analyzed for each individual is shown as is the total number of compounds found (excluding those also found in control samples). "Retention time" is defined as the time taken for each analyte to emerge from the chromatographic column (here given in minutes).

No.	Retention time	Name	Tiger males			Cubs		Tiger females			
			Intact			Castrates					
			Sym	Rub	Ram	Aba	Kac	Maj	Und	Vel	Fel
1	1.126	trimethylamine	X	X		X		X		X	X
2	1.342	pentane?	X								
3	1.376	pentane	0	0	0	0	0	0	0	0	0
4	1.409	?	X								
5	1.426	ethanol	X				X	X			
6	1.642	acetone	0	0	0	0	0	0	0	0	0
7	1.742	carbon disulfide	0	0	0	0	0	0	0	0	0
8	1.909	methylene chloride	0	0	0	0	0	0	0	0	0
9	2.093	methy/methylether	0			0		0	0		
10	2.292	hexane	X		0	0		0	0		
11	2.442	diisopropyl ether	0		0	0		0	0		
12	2.676/2.809	2-butanone	X	X	X	X	X	X	X	X	X
13	2.809	?	0					0			
14	3.026	tetrahydrofuran (THF) + chloroform	0			0		0	0		
15	3.243/3.610	3-methylbutanal	X	X	X		X	X			X
16	3.493	benzene + colum bleeding	0			0		0	0		
17	3.560	acetic acid + trichloroethylene	X								
18	3.593	2,2,3,3-tetramethylbutane				X			0		
19	3.693/4.293	2-pentanone	X			X	X	X	X		
20	3.693/3.776/4.360	3-methyl-1-butylamine	X	X	X	X		X		X	X
21	3.693/4.360/4.393	pentanal	X			X	X	X			
22	3.709/4.393	amine?							X		
23	3.726	2-methyl-butanol	X								
24	3.776	heptane							X		
25	3.793	?					X				
26	4.093	trichloroethylene	0			0		0	0		
27	4.260	s-methyl ester ethanethioic acid + 2-pentanone				X		X			
28	4.276	2-pentene				X			X		
29	4.376	n-ethyl-1-propylamine				X					
30	4.443	5-hydroxy-4-methyl-6-hepten-3-one		X	X	X			X	X	X
31	4.460/5.893	1-methoxyethanethiol	X			X	X	X	X		
32	4.576	bromodichloro-methane	0			0		0	0		
33	4.843	hexanal	X						X		
34	5.076	dimethyl-disulfide	X	X		X					X
35	5.393	3-methyl-1-butanol	X			X	X	X			
36	5.410	toluene	0			0		0	0		
37	5.493	3-methyl-2-pentanone				X	X				
38	5.593	octane	X								
39	5.743	3-methyl-pentane	X								
40	5.860	2-heptanone				X			X		
41	5.926/8.277	heptanal	X			X		X	X		
42	6.043	3-hexanone	X	X	X	X		X	X		
43	6.043	tetrachloroethylene	0			0			0		
44	6.110	cyclohexanone	0			0	0	0	0		
45	6.343	dibromochloromethane + hexanal				0		0	0		
46	6.343	hexanal (with contam.)	X				X	X			

No.	Retention time	Name	Sym	Rub	Ram	Aba	Kac	Maj	Und	Vel	Fel
47	6.477	?				0					
48	6.777/9.777	benzaldehyde	X		X	X	0	X	X		X
49	6.943/10.194/10.228	octanal	X					X	X		
50	7.094	chlorobenzene	0			0			0		
51	7.277	ethylbenzene	0			0			0		
52	7.427	p-xylene	0			0		0	0		
53	7.527	nonane	X								
54	7.627	1-octanal	X						X		
55	7.644	amine?						X			
56	7.727/7.760	4-heptanone	X			X			X		
57	7.894/11.995/12.045	nonanal	X			X		X	X		
58	7.960	o-xylene + styrene	0			0			0		
59	7.994	2,4-dithiapentane	X								
60	8.177	tribromomethane + 2-heptanone	0						0		
61	8.177	2-heptanone (or methyl-ketone)	X	X	X	X		X	X	X	X
62	8.760	decanal	X			X					
63	9.427	6-methyl-2-heptanone				X		X			
64	9.861	2,3-octanedione + benzaldehyde						X			
65	10.044	2-octanone	X								
66	11.278	phenol	X					X			
67	11.428	?	X			X		X	X		
68	11.811	1-phenyl-1-pentanone(C11H14O isomer)	X								
69	11.828	2-undecanone	X	X	X	X		X	X	X	X
70	12.045	nonanal + methylbenzoate			X	X	X	X			X
71	12.511	?				X		X			
72	12.695	4-methyl-phenol	X								
73	13.712	naphthalene									0
74	13.745	decanal						X			
75	14.495	alkane				X					
76	15.062	2-nonadecanone	X								X
77	15.462	2-methyl-naphthalene				0					0
78	18.162	1,1'-(1,3-phenylene)bis-ethanone	X			X	X		X		
79	18.546	1,4-dihydro,-1,4-Ethenonaphthalate									X
80	18.829	?				X					
Number of samples analyzed:			4	2	1	2	2	3	1	2	2
Total number of compounds found (excluding controls):			38	9	9	30	12	26	20	6	12

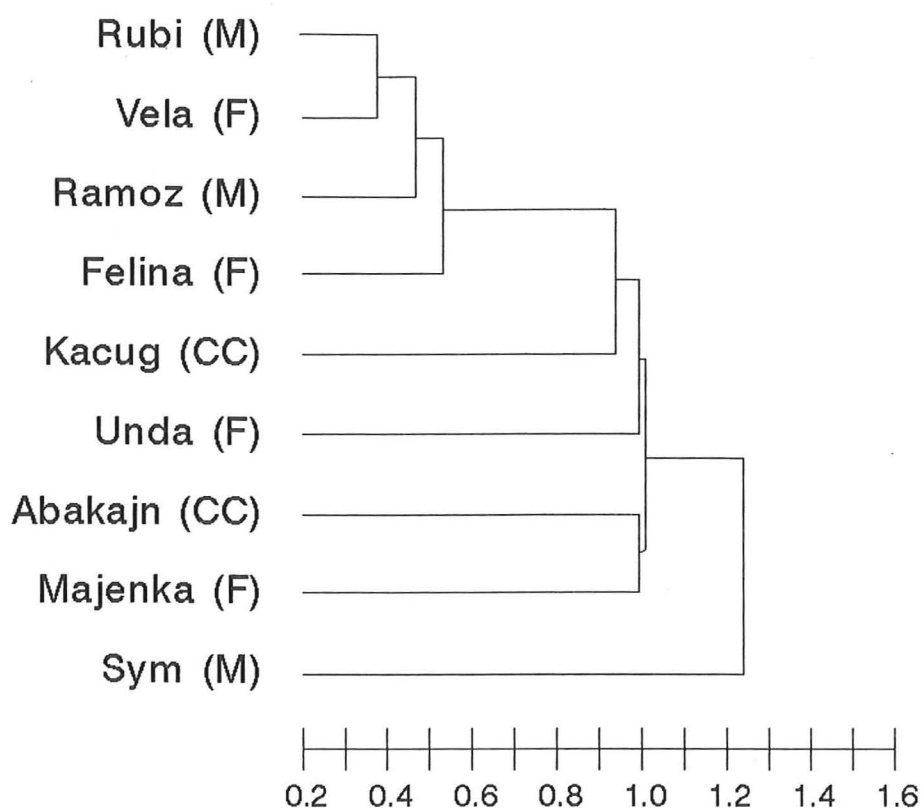


Figure 4.1.17: Average Linkage Cluster dendrogram showing the "relatedness" between individual tigers of the chemical composition of scent marks. The axis at the bottom shows the root-mean-square distance between observations.

M: male, F: female, CC: castrated cub.

Figure 4.1.18: Percentage overlap in compound composition within and between the tiger sex groups. M=males, C=castrates, F=females.

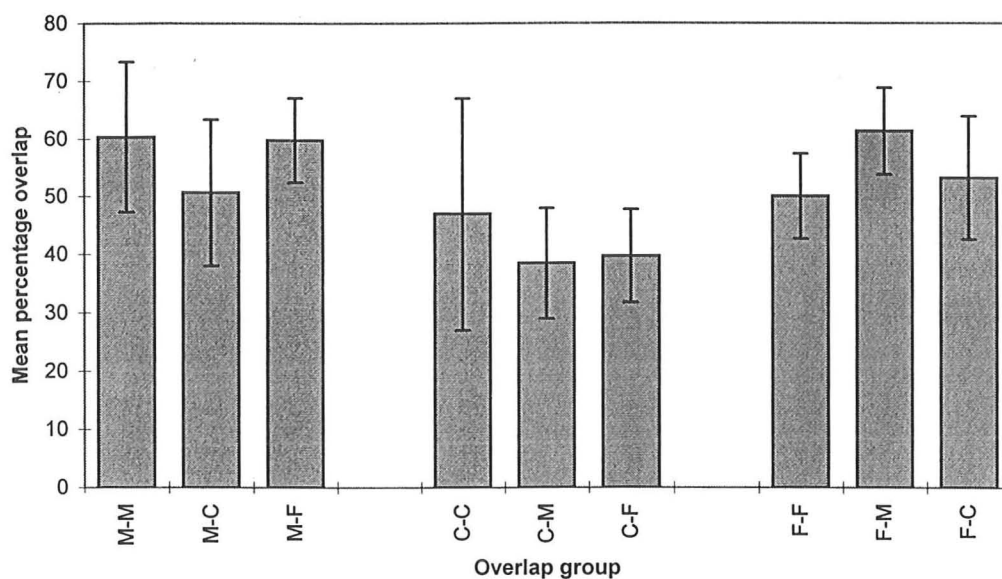


Figure 4.1.19: Percentage overlap in compound composition within and between individuals of the same sex group for tigers. M: male, CC: castrated cub, F: female

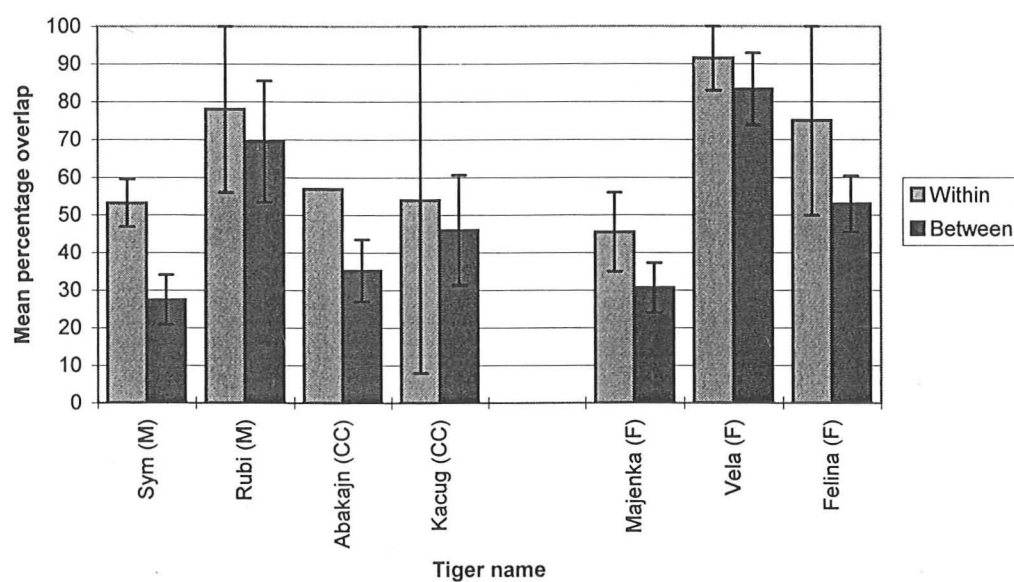


Figure 4.1.20: Sex difference in absolute concentration (area under peaks on chromatogram) for different compounds in tiger urine.

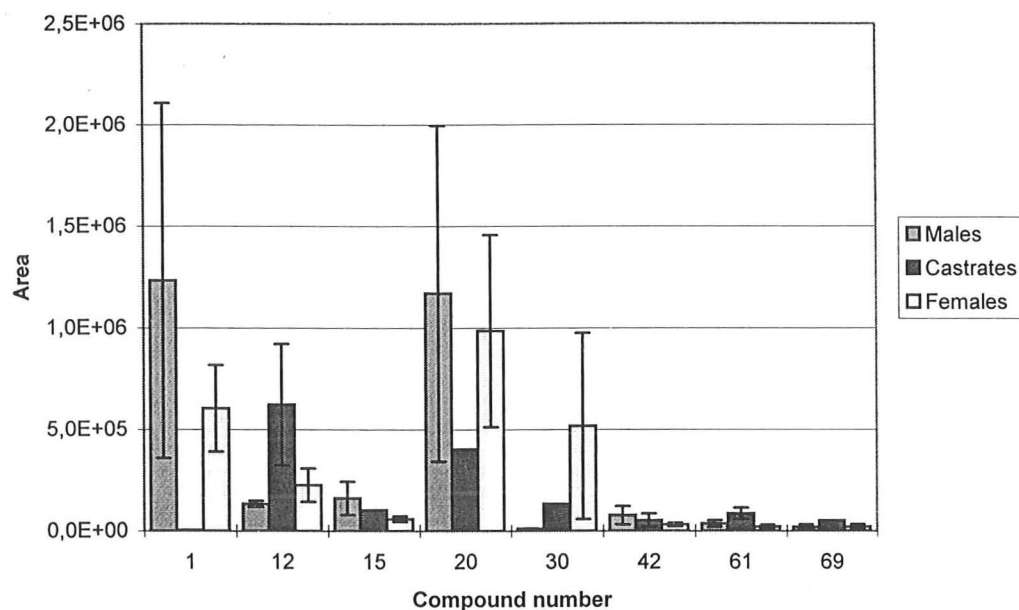


Figure 4.1.21: Sex difference in percentage concentration for different compounds in tiger urine.

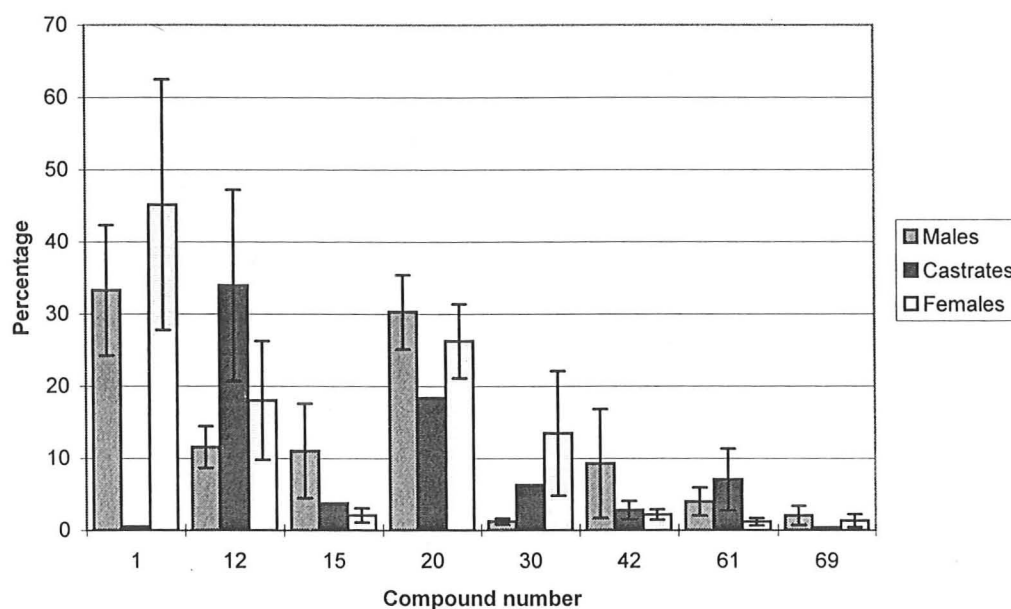


Figure 4.2.1: Activity of the KBH leopards.
M: male, F: female, C: cub.

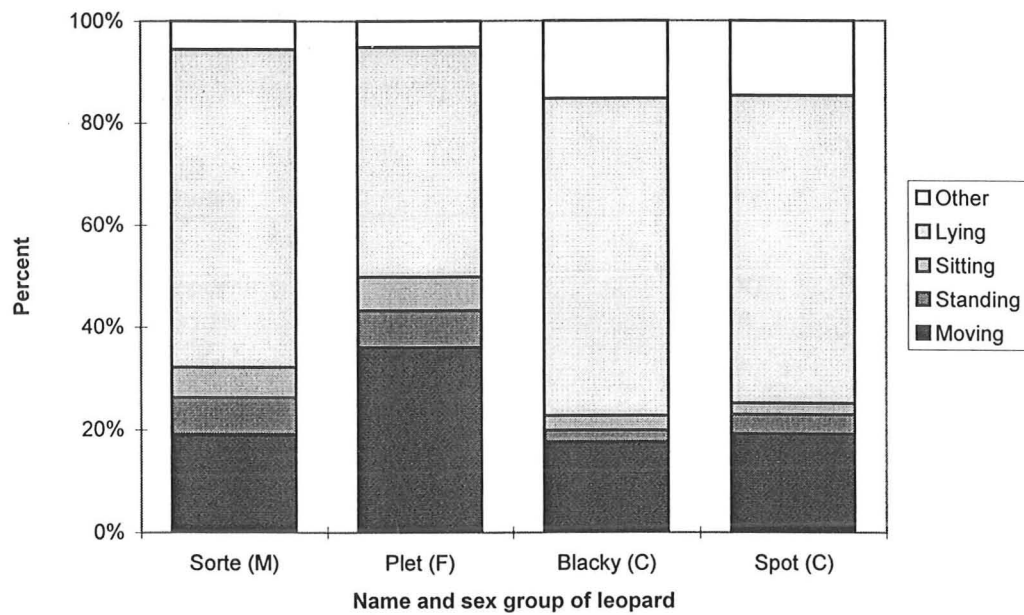
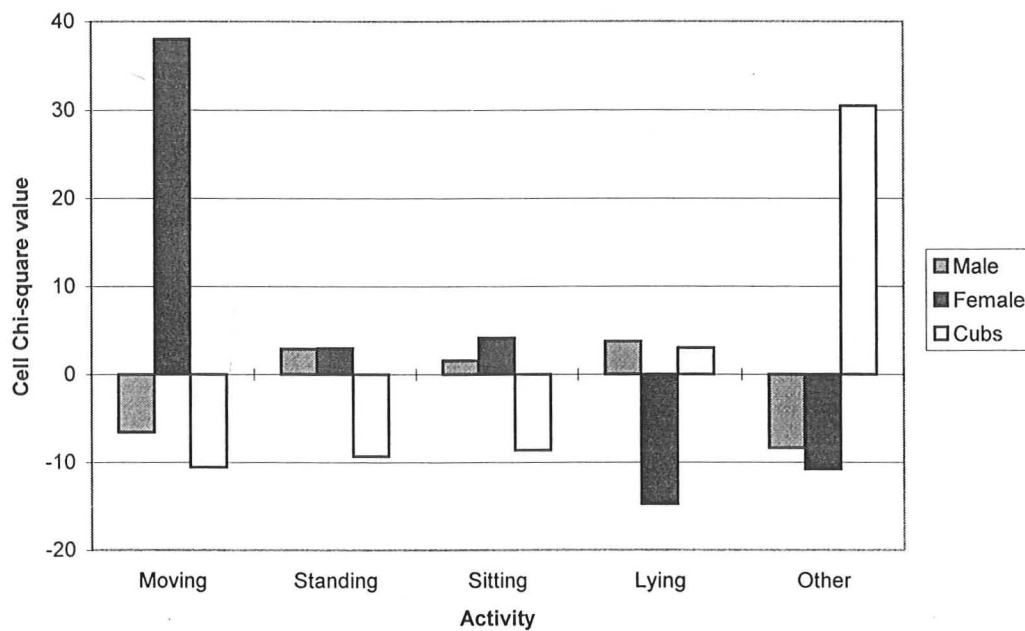


Figure 4.2.2: Activity differences between leopard sex/age groups.
Cell Chi-square values including direction of deviation.



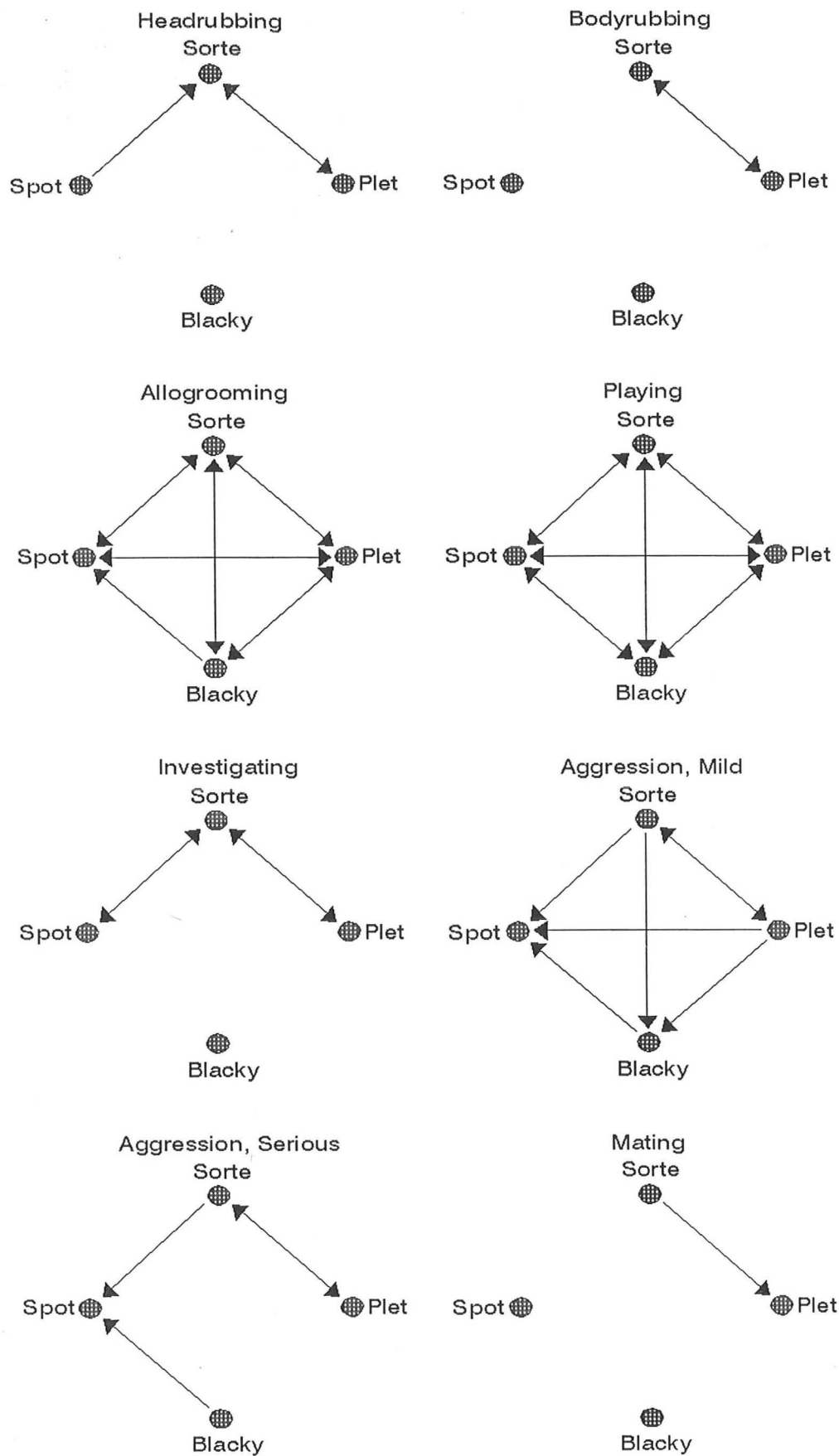


Figure 4.2.3: Qualitative social interactions between all individuals in the KBH leopard group. Arrows indicate direction of interactions from the Actor to the Target. M: Sorte, F: Plet, C: Blacky and Spot.

Figure 4.2.4: Percentage distribution of the six most frequent social behaviours performed by the KBH leopards.

M: male, F: female, C: cub.

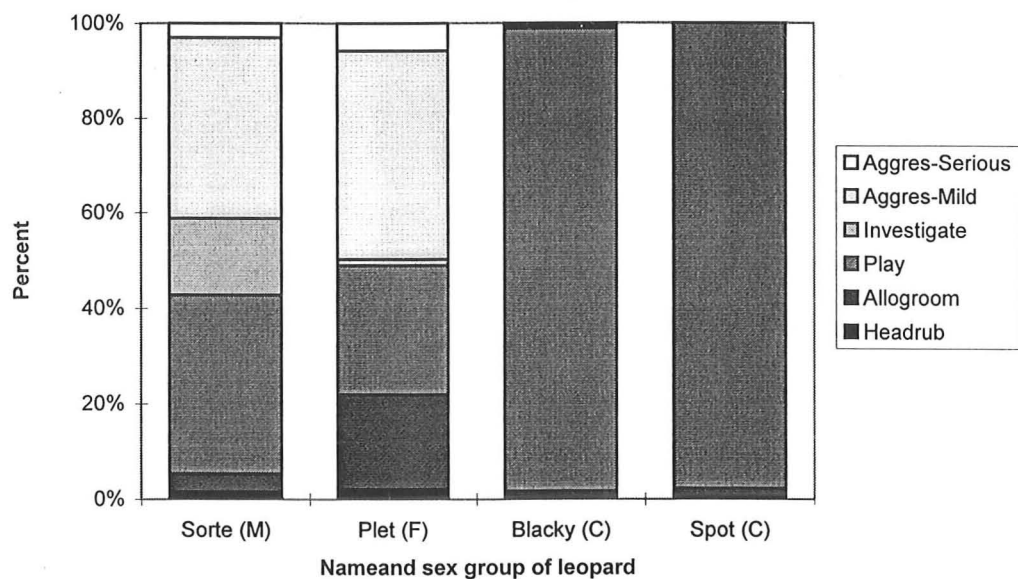


Figure 4.2.5: Social behaviour differences between leopard sex/age groups. Cell Chi-square values including direction of deviation.

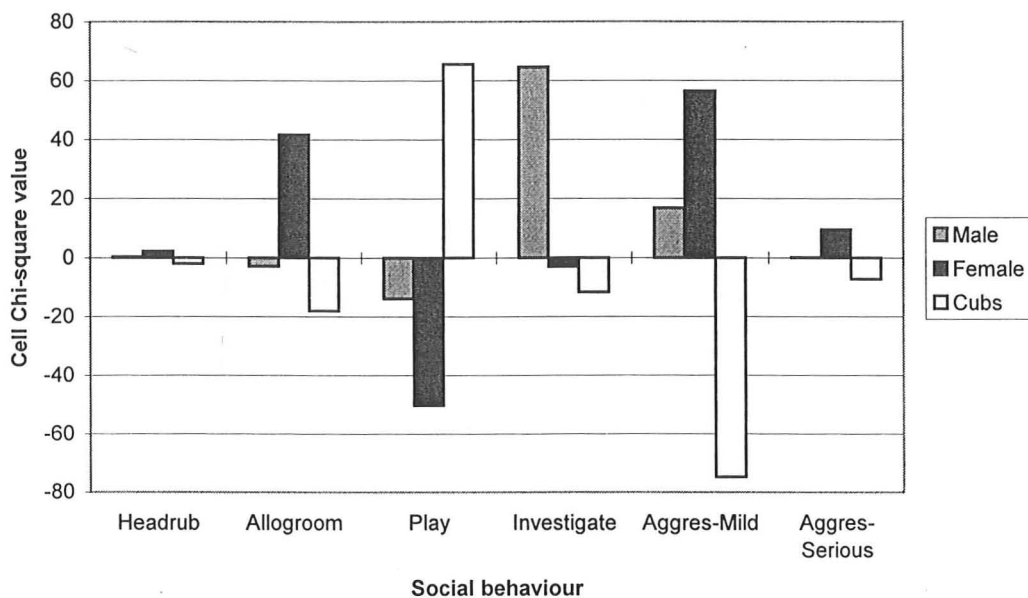


Figure 4.2.6: Number of Non-Aggressive and Aggressive social interactions between KBH leopard sex/age groups. Actor vs. Target groups are indicated below the x-axis.

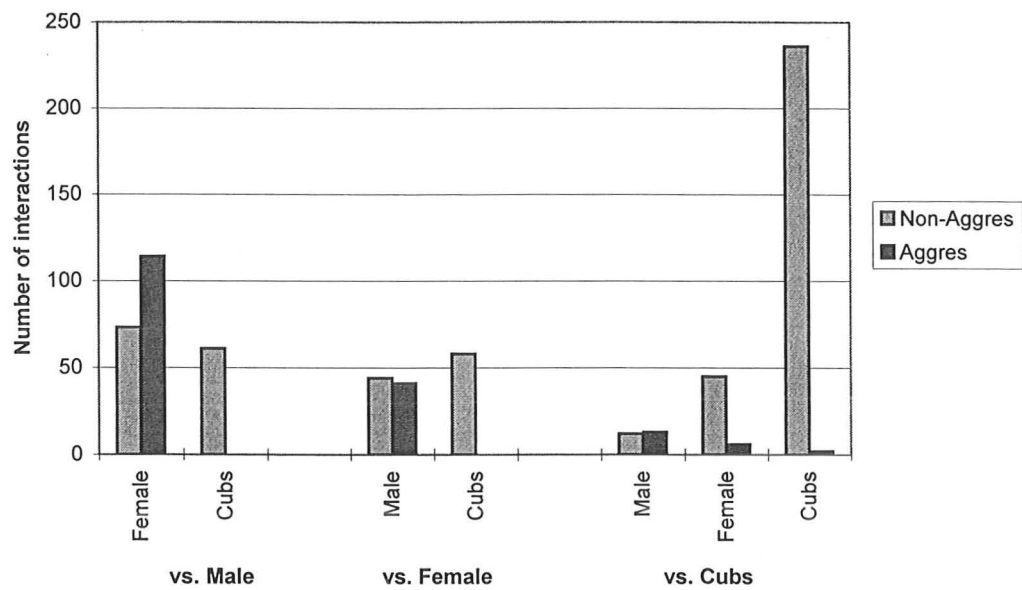


Figure 4.2.7: Percentage distribution of the different mark types for the male (M) and female (F) leopard in KBH.

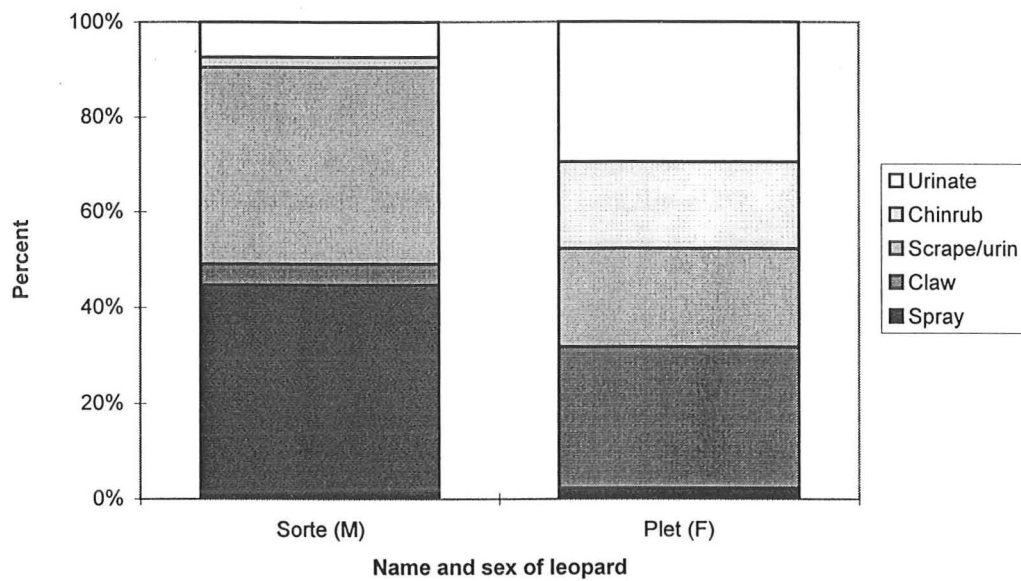


Figure 4.2.8: Marking rates of the different mark types for the KBH leopards. M: male, F: female.

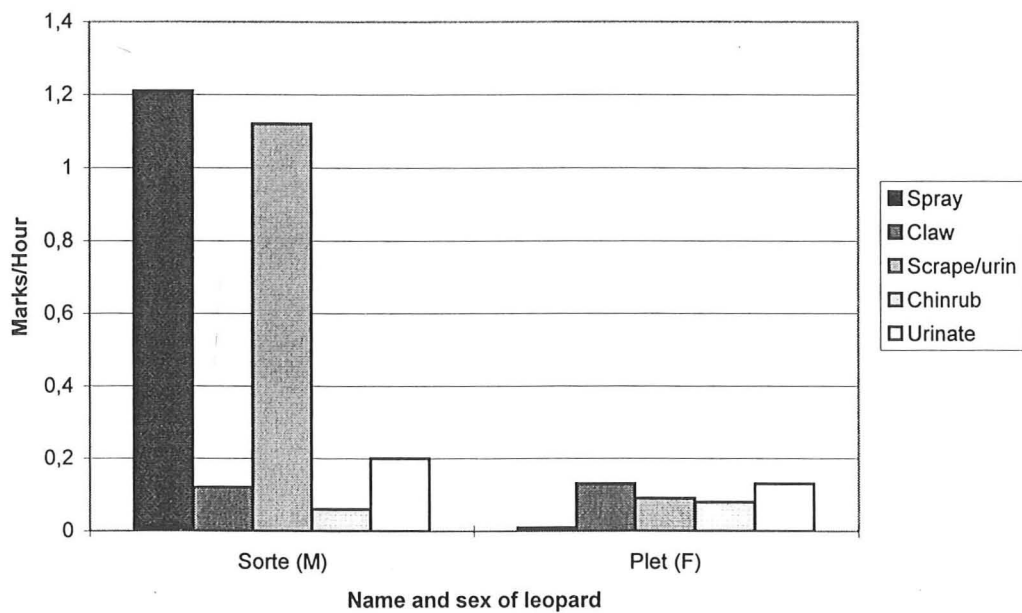


Figure 4.2.9: Response preference of leopard sex/age groups to different scent mark categories. Cell Chi-square values including direction of deviation.

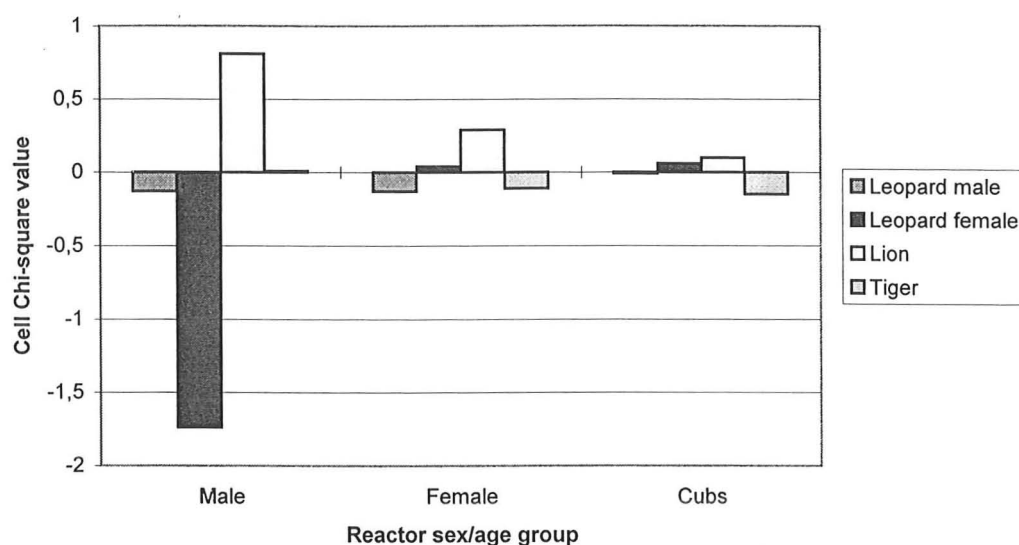


Figure 4.2 10: Frequency of Scentings (per 4 hrs presentations) for KBH leopards when investigating different experimental mark categories.

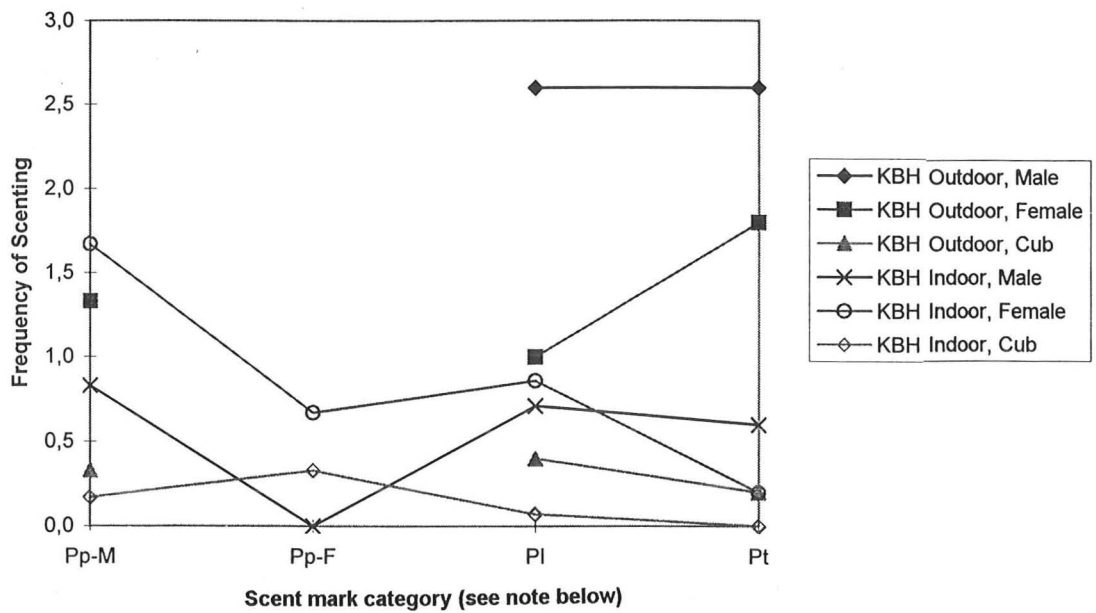
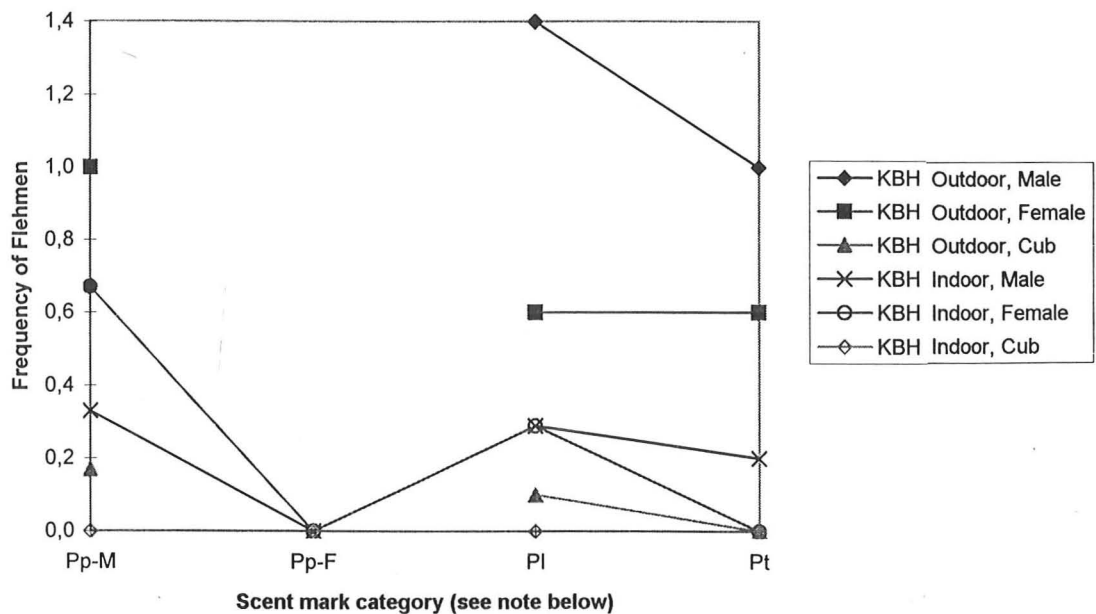


Figure 4.2.11: Frequency of Flehmens (per 4 hrs presentations) for KBH leopards when investigating different experimental mark categories.



Note to Figures 4.2.10 and 4.2.11:

Scent mark categories: Pp-M: Leopard male mark, Pp-F: Leopard female mark, PI: Lion mark, Pt: Tiger mark.

Table 4.2.1: Compounds present in the urine of male and/or female leopards (n=10). The "Unknown sex" group contains samples from both the male Sorte and the female Plet which were kept together in the cage where the samples were taken. "X" denote the presence of the compound in one or more of the samples analyzed. "0" indicates that the compound was present in both the sample and in a corresponding control sample. "Retention time" is defined as the time taken for each analyte to emerge from the chromatographic column (here given in minutes).

			Leopards					
			Males			Females		
Number	Retention time	Name	Sor	Tab		Ple	Urm	Unknown sex
1	1.126	trimethylamine		X				X
2	1.342	pentane?					X	
3	1.376	pentane		0		0	0	0
4	1.426	ethanol						X
5	1.642	acetone	0	0		0	0	0
6	1.742	carbon disulfide	0	0		0	0	0
7	1.909	methylene chloride	0	0		0	0	0
8	2.093	methylmethylether						0
9	2.292	hexane						0
10	2.442	diisopropyl ether						0
11	2.676/2.809	2-butanone	X	X				X
12	2.809	?						0
13	3.026	tetrahydrofuran (THF) + chloroform						0
14	3.493	benzene + colum bleeding						0
15	3.560	acetic acid + trichloroethylene						X
16	3.593	2,2,3,3-tetramethylbutane						0
17	3.693/4.360/4.393	pentanal						X
18	4.093	trichloroethylene						0
19	4.260	s-methyl ester ethanethioic acid + 2-pentanone						X
20	4.460/5.893	1-methoxyethanethiol						X
21	4.576	bromodichlore-methane						0
22	5.410	toluene						0
23	6.043	tetrachloroethylene						0
24	6.110	cyclohexanone						0
25	6.343	dibromochloromethane + hexanal						0
26	6.477	?						0
27	6.777/9.777	benzaldehyde						X
28	7.094	chlorobenzene						0
29	7.427	p-xylene						0
30	7.727/7.760	4-heptanone						X
31	7.894/11.995/12.045	nonanal						X
32	7.960	o-xylene + styrene						0
33	8.177	2-heptanone (or methyl-ketone)						X
34	11.211	alkane						X
35	11.811	1-phenyl-1-pentanone(C11H14O isomer)		X				X
36	12.045	nonanal + methylbenzoate	X			X		X
37	12.912	dodecane						X
38	14.495	alkane						X
39	20.947	diethyl phthalate						X
Number of samples analyzed:			1	2		1	1	5
Number of compounds found excluding controls:			2	3		1	1	17



CHAPTER 5: INTERSPECIFIC COMPARISON OF SCENT MARKING AND MARKING FLUID.

In this chapter the results presented in the two previous chapters on lions, tigers and leopards are analysed from an interspecific perspective. This will allow further testing of the hypotheses listed in the Introduction as well as generalisations across species on issues of scent marking behaviour and chemical composition of scent marks. The figures and tables referred to in the text are found between pages 172 and 178.

5.1 Scent marking behaviour.

The three *Panthera* species included in the present study used the same range of scent marking behaviours in their marking repertoire, i.e. *Spraymarking*, *Scrape/urination*, *Chinrubbing*, *Clawing* and *Urination*. In this section the two most common types of scent marking, *Spraymarking* and *Scrape/urination*, will be compared for the three focal species. The comparison will involve both the percentage of the total marking repertoire which each of them make up, and also the marking rates. These results will provide further data which can be used in the evaluation of Hypotheses 1, 2 and 3.

It must again be stressed that parts of the data are based on only one individual and the conclusions involving these data should therefore be seen only as indications of possible trends. The species and sex groups with a sample size of one are intact tiger male and the male and female leopards.

Results.

Percentage of *Spraymarking*.

The mean percentages of *Spraymarking* in the total scent marking repertoire for all species and sex groups are presented in **Figure 5.1**. The leopard female had the lowest percentage of *Spraymarking* followed by the lion females. The highest percentage was found for the tiger male. Only one significant difference was found in the interspecific comparison, namely that the tiger females had a significantly higher mean percentage of *Spraymarking* than the lion females ($t=2.20$, $DF=12$, $P=0.048$).

Percentage of *Scrape/urination*.

In **Figure 5.2** the mean percentage of *Scrape/urination* for all species sex groups is shown. The lowest values are found for the intact tiger male and for the castrated tiger males, whereas the highest value is seen in the leopard male. The following interspecific differences were revealed in the comparative analysis. The lion males had significantly higher mean percentage of *Scrape/urination* than the tiger male ($t=3.87$, $DF=4$, $P=0.018$), the tiger females ($t=4.22$, $DF=7$, $P=0.004$) and the castrated tiger males ($t=6.75$, $DF=6$, $P<0.001$). The lion females had a significant higher value than the castrated tiger males (unequal variance, $t=4.35$, $DF=9.8$, $P=0.0015$). The leopard male had higher values than the tiger females ($t=3.23$, $DF=3$, $P=0.048$) and the castrated tiger males ($t=11.80$, $DF=2$, $P=0.007$). Finally, the leopard female had a higher value than the castrated male tigers ($t=5.50$, $DF=2$, $P=0.032$).

Rates of *Spraymarking*.

The mean rate of *Spraymarking* for the different species sex groups is depicted in **Figure 5.3**. The lowest values were found for the leopard female, the castrated tiger males and the lion females. The highest value were seen in the tiger females and male and the leopard male. Three significant interspecific differences were found. These

were the higher rate of *Spraymarking* of the tiger male compared to the lion females ($t=14.14$, $DF=9$, $P<0.001$), and the higher rate of the leopard male compared to both the lion females ($t=14.39$, $DF=9$, $P<0.001$) and the castrated tiger males ($t=22.11$, $DF=2$, $P=0.002$).

Rates of *Scrape/urination*.

Figure 5.4 show the mean rate of *Scrape/urination* for the different species sex groups. The lowest values were found for the castrated tiger males and for the intact tiger male. The leopard male had by far the highest rate of *Scrape/urination*. Several significant differences were found in the interspecific comparison. The lion males and females both had significantly higher rates than the castrated tiger males (males: unequal variance, $t=6.89$, $DF=4$, $P=0.002$; females: unequal variance, $t=2.84$, $DF=9.6$, $P=0.018$), and significantly lower rates than the leopard male (males: $t=5.81$, $DF=4$, $P=0.004$; females: $t=18.34$, $DF=9$, $P<0.001$). The leopard male had a significantly higher rate than both the tiger females ($t=3.39$, $DF=3$, $P=0.043$) and the castrated tiger males ($t=167.50$, $DF=2$, $P<0.001$). The leopard female had a higher rate than the castrated tiger males ($t=13.00$, $DF=2$, $P=0.006$).

Discussion.

As pointed out in the Introduction, the three species studied during this project are characterised by differences in social organisation which have implications for the way in which the scent marking behaviour for each species can be optimised.

According to Hypothesis 2 the lion females, which defend a group territory, should have lower marking rates than both the tiger and the leopard females, which both defend individual territories. A further prediction from Hypothesis 2 is that tiger females and leopard females should have comparable levels of scent marking. Starting with the last prediction first, it appears to hold quite well as no significant differences were found between tiger and leopard females for either *Spraymarking* or *Scrape/urination*. This was true for both the rates and the percentage distribution. However, for *Spraymarking* the leopard female had both a lower rate and a lower

percentage distribution than the tiger females even though they were not significant. Going back to the first prediction i.e. that the lion females should have lower levels than tiger and leopard females, the following are revealed from the figures. The tiger females did indeed have higher rates of both *Spraymarking* and *Scrape/urination* than the lion females, though not significantly so. The leopard female had a higher rate of *Scrape/urination* but a lower rate of *Spraymarking* than the lion females. The same differences were not reflected in the percentage repertoire of the females. Here the tiger females had a significantly higher value than the lion females for *Spraymarking*, but a lower value for *Scrape/urination*. The leopard female had lower values than the lion females in both mark categories. It thus appears that Hypothesis 2 generally holds for the marking rates and that the picture is more complicated and less predictable for the percentage distribution.

The males of the three species all hold relatively large territories and one would therefore expect them to have equal levels of scent marking (indirect prediction from Hypothesis 1). This was indeed the general case. No significant differences were found between the *Spraymarking* rates of the males, and for the *Scrape/urination* rates only the leopard male rate was significantly higher than the lion male rate. However, the tiger male rate of *Scrape/urination* was much lower than the leopard male rate, though again it should be remembered that there is only one individual in each of these two groups. For the percentage distribution only one significant difference was found between the male groups, namely the higher percentage of *Scrape/urination* for the lion males when compared to the tiger male.

Hypothesis 3 predicts that the castrated tiger males should have lower marking rates than the intact males. This prediction should also hold on an interspecific level since intact males of the three species should have comparable levels, as was pointed out in the above paragraph. This was indeed the case for marking rates where the rates of *Spraymarking* of castrates were much lower than both lion males and leopard male, and the last significantly so. For *Scrape/urination* castrates had significantly lower rates than both lion males and leopard male. Castrates had comparable levels of percentage distribution of *Spraymarking* to the other two male groups, but their percentage distribution of *Scrape/urination* was significantly lower than both the other two groups. Thus castration does seem to lower the general rates of marking as well

as the percentage distribution of *Scrape/urination*, whereas it does not seem to affect the percentage distribution of *Spraymarking* in the overall marking repertoire.

The remaining two *Panthera* species which were not included in the present study, the jaguar and the snow leopard, have social organisations comparable to tigers and leopards, i.e. they are solitary animals with each sex defending an intra-sex territory. Therefore Hypotheses 1 and 2 would predict that the marking rates of the sex groups of these two species should be similar to those of tigers and leopards. No quantitative data on scent marking for jaguars or snow leopards have been published so it is impossible to say at present if this prediction holds.

5.2 Chemical composition of scent marks.

This section compares the results on the chemical analysis of urine samples from lions, tigers and leopards on the interspecific level to identify common trends and differences in the chemical composition of the scent marks of the three species. The results presented are used in the evaluation of Hypothesis 8 and more indirectly of Hypotheses 5, 6 and 7.

Results.

A table of all the compounds identified in any of the three focal species is presented as **Table 5.1**. In the individual species tables of the previous two chapters the left column of numbers is not identical for the different tables. Therefore it is recommended that the Retention time values given in the second column should be used when comparing compounds between the tables. **Table 5.2** provide summary statistics of the overlap in compound composition between the species.

Eight of the 103 compounds listed in Table 5.1 were found in all species and all sex groups, including castrates. These were compounds 1 (trimethylamine), 5 (ethanol), 13 (2-butanone), 33 (1-methoxyethanethiol), 52 (benzaldehyde), 61 (4-heptanone), 63 (nonanal) and 68 (2-heptanone or methyl-ketone).

From Table 5.2 it is clear that 33% of the 58 compounds found in lions were unique to lion samples. These 19 compounds were number 3, 6, 7, 15, 35, 39, 51, 55, 62, 64, 69, 71, 74, 76, 78, 81, 88, 89 and 99. The five compounds shown in bold type, acetone (7), 1-pentene (35), 2-pentylfuran (51), 1,2-cyclooctanediene (69) and diethylbenzene (89), are compounds which were common to almost all the lion samples and which could therefore play a role as species identifying compounds.

19 compounds out of the total of 57 were specific to tiger samples. The numbers of these 19 compounds are 4, 20, 24, 30, 31, 40, 41, 58, 60, 66, 70, 73, 80, 85, 86, 92, 95, 98 and 102. None of these compounds was common to all tiger samples.

All the compounds found in the leopard samples were also identified in samples from either lion or tiger.

The overall overlap in compound composition for individual samples within and between the species was calculated and is depicted in **Figure 5.5**. An overlap is defined as the percentage of compounds common to two samples from the point of view of each of the samples i.e. if a lion sample containing 20 compounds and a tiger sample containing 15 compounds have 10 compounds in common, then the overlap from the lion's point of view will be 50% and from the tiger's 67%.

From the left part of Figure 5.5 it is clear that tiger samples overlapped significantly more within their own species than they did with either lion samples (unequal variance, $t=8.50$, $DF=82.4$, $P<0.001$) or leopard samples (unequal variance, $t=3.69$, $DF=51.1$, $P<0.001$). Similarly, from the middle part of the figure it can be seen that lion samples also overlapped significantly more within their own species than they did with either tiger samples (unequal variance, $t=14.15$, $DF=392.7$, $P<0.001$) or leopard samples (unequal variance, $t=7.82$, $DF=31.1$, $P<0.001$). Both lions and tigers overlapped significantly more with leopards than they did with each other (lions: unequal variance, $t=2.93$, $DF=34.5$, $P=0.006$; tigers: $t=3.61$, $DF=169$, $P<0.001$). Leopards did not overlap more within their own species than they did with lions or tigers, and there was no significant difference in the mean overlap of leopard with lion and that of leopard with tiger.

To complete the picture of similarities and differences in chemical composition of urine between the three *Panthera* species a cluster analysis was performed. The resulting dendrogram is shown in **Figure 5.6**. As is evident there is quite a strong

separation between the species though it is not perfect. The three top clusters, indicated by dotted boxes around the names, are composed of lions only, and they comprise all but two of the lions. These two male lions (Napoleon and KolmHan) are found in their own small cluster (number four from the top) and are part of the second big cluster from the top, indicated by empty dotted boxes on the far left, among part of the tiger individuals. The leopards are found in the sixth of the small right side clusters together with two tigers.

Discussion.

There are both differences and similarities between scent samples from different individuals and species and these can be indicators of the compounds which may be important in communication in these species. The origin of the compounds identified in scent samples are different, some will originate from purely metabolic processes, some may be hormone dependent and some may have still other origins (Albone 1984). If the diets of the animals in question are the same then the likelihood that some of the common compounds originate from the food is increased. All the animals in the present study were fed on mainly beef and consequently some common compounds may result in the marking fluid.

Eight of the compounds identified in the three *Panthera* species were present in all sex groups of all the species. However, they were not all found in the majority of the individual urine samples. This was only true for trimethylamine (No. 1), 2-butanone (No. 13) and nonanal (No. 63), if one excludes those also found in control samples of laboratory air and sawdust-extractions. These three compounds may convey a message of belonging to the genus *Panthera* either on their own or in combination with other as yet unidentified compounds. For this to be true they should also be present in the urine of the two non-focal *Panthera* species snow leopard and jaguar, and this still remains to be seen. Their origin, dietary or otherwise, is not known at present.

On a species level some candidates for common species identifying compounds were found for lions. The five compounds were acetone (7), 1-pentene (35), 2-pentylfuran (51), 1,2-cyclooctanediene (69) and diethylbenzene (89). One or more of these compounds alone could act as species identifying compounds, or they may work

in unison with the other 14 lion specific compounds to make up a bigger pool of lion compounds, where the presence of either one would be sufficient for identification.

None of the 19 compounds specific to tiger samples was present in all the samples analysed, so if any of them are important in species identification they would have to be part of a larger pool of tiger compounds, in which the presence of any one of the compounds alone or in combination could convey a message of species identity. However, other common compounds which have avoided detection in this study may be present, in which case they alone or in combination with the 19 compounds may function as species identifiers.

Leopard urine samples were the least complex of the three species. They contained only about a third of the number of compounds present in lion and tiger samples. The leopards studied here shared all their compounds with either lion or tigers leaving no candidates for species identifying compounds in the ones found here. This could have two reasons. The first being that there might be additional as yet unidentified compounds which can work as species identifiers. The second reason is more speculative. The leopard is sympatric with both the lion and the tiger but the latter two species are not sympatric. Therefore in areas where leopards overlap with lions, the compounds they share only with tigers could be used to distinguish their scent marks from those of lions. Similarly, in areas where leopards and tigers overlap, the compounds which leopards share only with lions could distinguish their marks from tiger marks. In this way acetic acid and trichloroethylene (No. 19) could work as species identifying compounds for the leopard in Africa, whereas the unidentified alkane (No. 77), dodecane (No. 87) and diethyl phthalate (No. 103) could be the leopard identifying compounds in Asia. However, more leopard samples need to be analysed in greater detail before this argument can be accepted.

It seems likely that the overall compound composition could play a role at the level of species identification for at least lions and tigers. This statement is based on the results that both lion and tiger samples overlapped significantly more in compound composition with other samples from their own species than they did with samples from any of the two other species. Consistent differences in concentration of specific compounds could provide further grounds for distinction between the species.

In all three *Panthera* species there was evidence of a high variability in compound composition as well as in concentration in samples from the same individual taken on

different days. Unfortunately this phenomenon could not be investigated in detail in the present study, but it would be worthwhile to investigate this further in the future, especially to see if the fluctuations are purely random or whether they contain elements of periodicity or seasonality.

Very few other results of the chemical composition of urine for other species are available in the literature. A comparisons with these other species show that several compounds in *Panthera* urine are common with those in the urine of the wolf (Raymer *et al.* 1986) and mouse (Schwende *et al.* 1986) (Table 5.3). It is evident that the highest overlap in compound composition is found between lion, tiger and wolf urine. Lion and wolf urine share 12 compounds plus three which were also found in lion control samples. Tiger and wolf share ten compounds plus five which were also seen in control samples. Leopard shared only four compounds with wolf plus an additional five which were also found in control samples. Mouse urine share only eight compounds with lion and tiger urine and of these eight compounds, two were also found in control samples for lions and four for tigers. Leopard urine share five compounds with mouse urine, four of which were also found in controls. The general higher overlap in compound composition of the *Panthera* species with wolf than mouse could possibly be related to their common carnivorous nature. This would mean that the common compounds would be of dietary origin, but this still remains to be verified.

Raymer *et al.* stated that the presence of diethyl phthalate (No 103) was an artifact resulting from the method used. Three compounds were found in the urine of all five species, namely acetone (No 7), toluene (No 39) and benzaldehyde (No 52).

5.3 Summary.

The differences in social organization between lions and tigers and leopards should lead to detectable differences in their scent marking behaviour. For the tigers and leopards in this study the number of individuals in some of the sex/age categories were very low and down to one in some cases. Therefore the comparisons involving these groups are preliminary and can give only indications of whether support for the different hypotheses are found.

It was found that tiger females had a significantly higher percentage of *Spraymarking* in their marking repertoire than female lions. Both lion males and females had significantly higher percentages of *Scrape/urination* than the castrated tiger males, and the lion males also had a significantly higher percentage than the intact tiger male and the tiger females.

Both the tiger male and the leopard male had a significantly higher rate of *Spraymarking* than the lion females. The lion males and females both had significantly higher rates of *Scrape/urination* than the castrated tiger males, and so did the leopard male and female.

Eight chemical compounds were found to be common to urine samples from all three species. 33% of the compounds found in lion or tiger samples were unique to either species whereas leopard shared all compounds with either lion or tiger.

The mean overlap of lion samples with other lion samples was significantly higher than the mean overlap between lion and tiger or lion and leopard samples. Tiger samples also had a significantly higher "within species" overlap than "between species" overlap. A similar relationship was not found for leopards.

All three *Panthera* species overlapped more in compound composition with another carnivore species (wolf) than they did with a rodent (mouse).

Figure 5.1: Percentage of Spraymarking for sex groups of the three *Panthera* species.

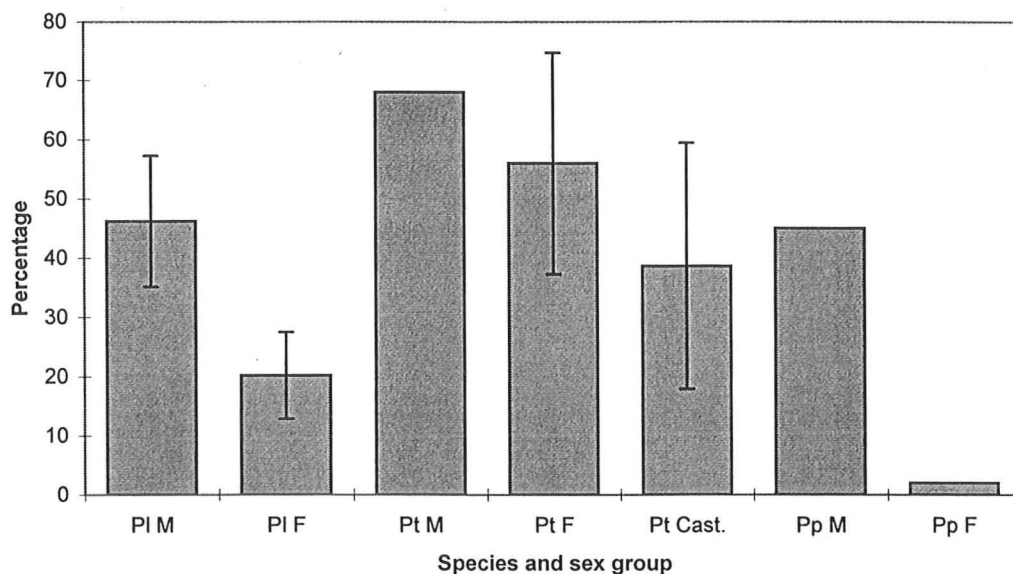
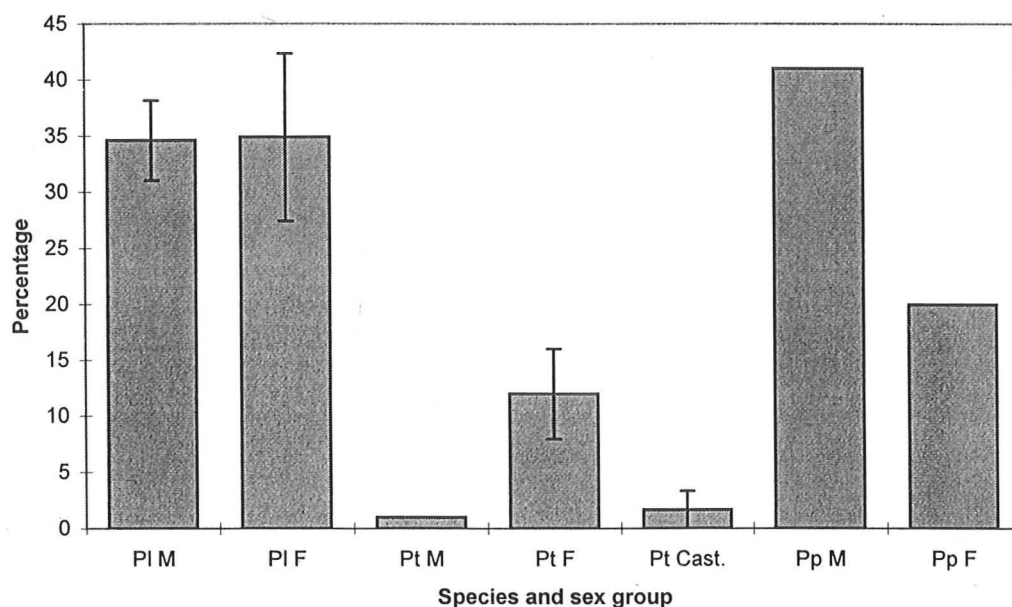


Figure 5.2: Percentage of Scrape/urination for sex groups of the three *Panthera* species.



Note to Figures 5.1 and 5.2:

Species and sex group categories:

PI M: Lion male, PI F: Lion female, Pt M: Tiger male, Pt F: Tiger female,

Pt Cast: Tiger castrated adult male, Pp M: Leopard male, Pp F: Leopard female.

Figure 5.3: Rates of Spraymarking for the sex groups of the three *Panthera* species.

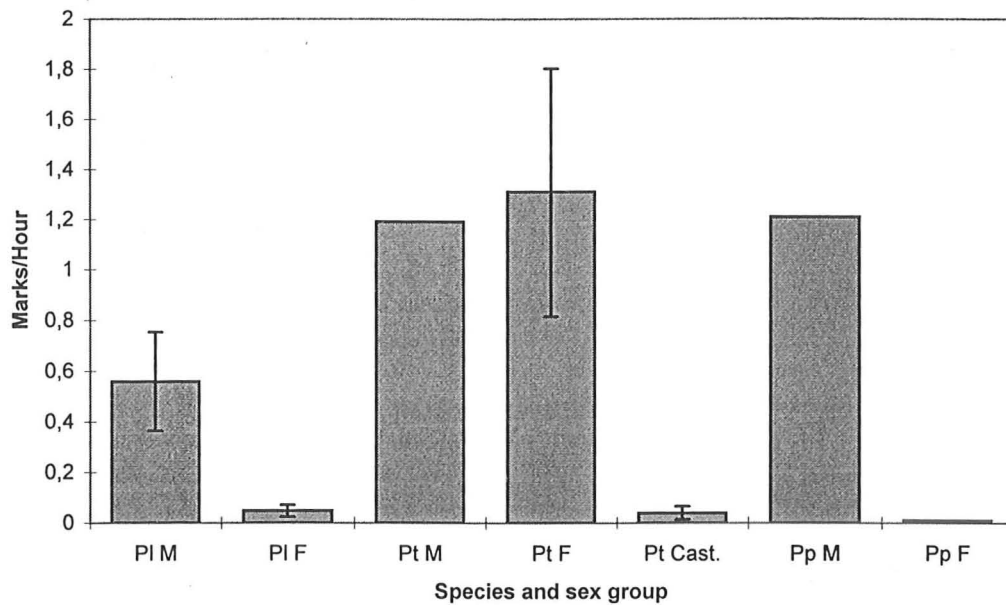
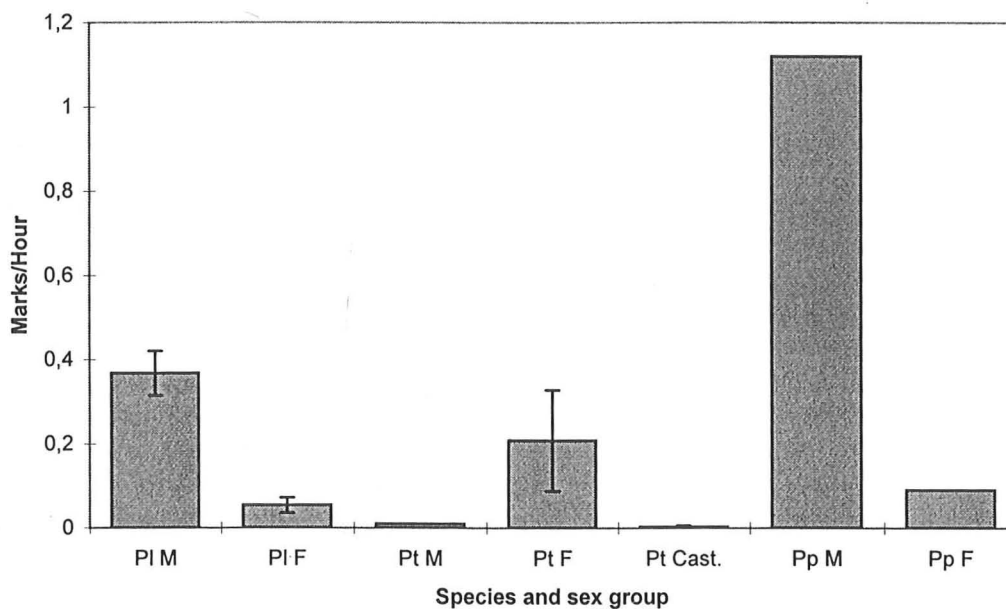


Figure 5.4: Rates of Scrape/urination for sex groups of the three *Panthera* species.



Note to Figures 5.3 and 5.4:

Species and sex group categories:

PI M: Lion male, PI F: Lion female, Pt M: Tiger male, Pt F: Tiger female,

Pt Cast: Tiger castrated adult male, Pp M: Leopard male, Pp F: Leopard female.

Table 5.1: The presence of compounds in urine samples from lions (n=63), tigers (n=19) and leopards (n=10). Lions and tigers are subdivided into males and females, and tigers also include castrated male cubs. "X" denotes the presence of the compound in one or more of the samples analyzed. A "0" indicates that the compound was present in both the sample and in a corresponding control (laboratory air) sample, and a "S" indicate that the compound was found in both the sample and in the "water-sawdust" extraction (only for lions). "Retention time" is defined as the time taken for each analyte to emerge from the chromatographic column (here given in minutes).

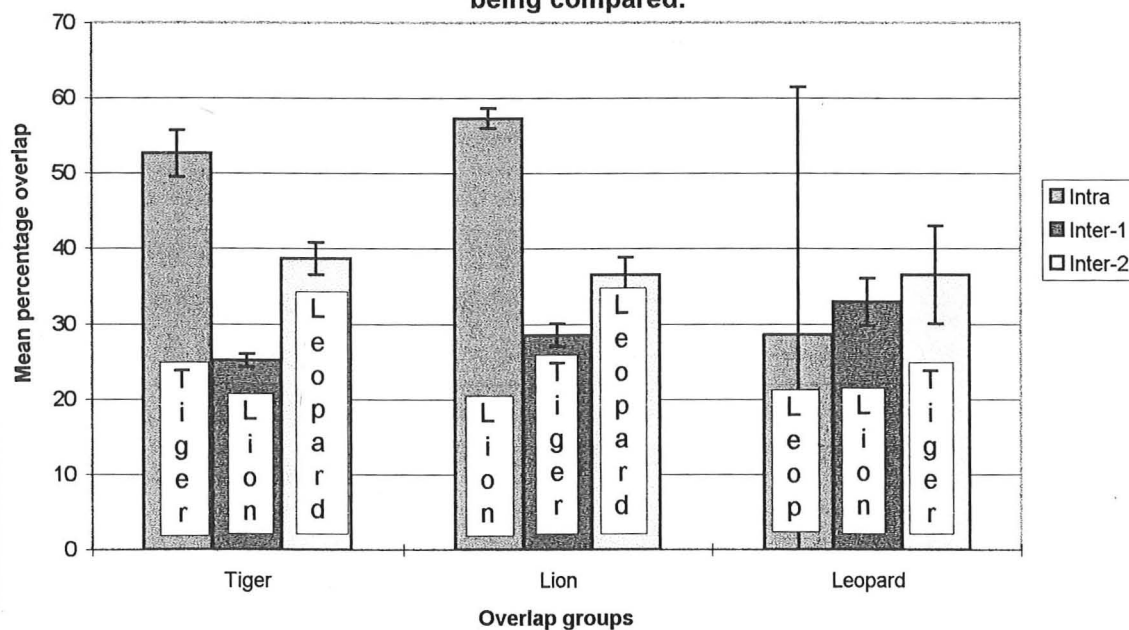
No.	Retention time	Name	Lion		Tiger			Leop.
			Male	Female	Male	Castr.	Female	
1	1.126	trimethylamine	X	X	X	X	X	X
2	1.342	pentane?			X			X
3	1.376	pentane	0	X	0	0	0	0
4	1.409	?			X			
5	1.426	ethanol	X	X	X	X	X	X
6	1.592	azetidine?		X				
7	1.642	acetone	X	X	0	0	0	0
8	1.742	carbon disulfide	0	0	0	0	0	0
9	1.909	methylene chloride	0	0	0	0	0	0
10	2.093	methylmethylether	0	0	0	0	0	0
11	2.292	hexane	X	X	X	0	0	0
12	2.442	diisopropyl ether	0	0	0	0	0	0
13	2.676/2.809	2-butanone	X	X	X	X	X	X
14	2.809	?			0		0	0
15	2.993	(+/-)-2-butanol		X				
16	3.026	tetrahydrofuran (THF) + chloroform	0	0	0	0	0	0
17	3.243/3.610	3-methylbutanal	X	S	X	X	X	
18	3.493	benzene + colum bleeding	0	0	0	0	0	0
19	3.560	acetic acid + trichloroethylene			X			X
20	3.593	2,2,3,3-tetramethylbutane	0	0		X	0	0
21	3.693/4.293	2-pentanone	X	X	X	X	X	
22	3.693/3.776/4.360	3-methyl-1-butylamine	X		X	X	X	
23	3.693/4.360/4.393	pentanal	S	X	X	X	X	X
24	3.709/4.393	amine?					X	
25	3.726	2-methyl-butanol	X	X	X			
26	3.776	heptane		X			X	
27	3.793	?	X			X		
28	4.093	trichloroethylene	0	0	0	0	0	0
29	4.260	s-methyl ester ethanethioic acid + 2-pentanone		X		X	X	X
30	4.276	2-pentene				X	X	
31	4.376	n-ethyl-1-propylamine				X		
32	4.443	5-hydroxy-4-methyl-6-hepten-3-one	X	X	X	X	X	
33	4.460/5.893	1-methoxyethanethiol	X	X	X	X	X	X
34	4.576	bromodichloro-methane	0	0	0	0	0	0
35	4.610	1-pentene	X	X				
36	4.843	hexanal	X	X	X		X	
37	5.076	dimethyl-disulfide	X	X	X	X	X	
38	5.393	3-methyl-1-butanol		X	X	X	X	
39	5.410	toluene	X	X	0	0	0	0
40	5.493	3-methyl-2-pentanone				X		
41	5.593	octane			X			
42	5.743	3-methyl-pentane		X	X			
43	5.860	2-heptanone		X		X	X	
44	5.926/8.277	heptanal	X	X	X	X	X	
45	6.043	3-hexanone	X	X	X	X	X	
46	6.043	tetrachloroethylene	0	0	0	0	0	0
47	6.110	cyclohexanone	0	0	0	0	0	0
48	6.343	dibromochloromethane + hexanal		0		0	0	0

No.	Retention time	Name	Lion		Tiger			Leop.
			Male	Female	Male	Castr.	Female	
49	6.343	hexanal (with contam.)	S	X	X	X	X	
50	6.477	?				0		0
51	6.610	2-pentylfuran	X	X				
52	6.777/9.777	benzaldehyde	X	X	X	X	X	X
53	6.943/10.194/10.228	octanal	X	X	X		X	
54	7.094	chlorobenzene	0	0	0	0	0	0
55	7.260	4-methyl-3-penten-2-one		X				
56	7.277	ethylbenzene	0	0	0	0	0	
57	7.427	p-xylene	0	0	0	0	0	0
58	7.527	nonane			X			
59	7.627	1-octanal	X	X	X		X	
60	7.644	amine?					X	
61	7.727/7.760	4-heptanone	X	X	X	X	X	X
62	7.744	2-n-butyl-furan	X	X				
63	7.894/11.995/12.045	nonanal	X	X	X	X	X	X
64	7.927	3-methyl-1-pentanol	X	X				
65	7.960	o-xylene + styrene	0	0	0	0	0	0
66	7.994	2,4-dithiapentane			X			
67	8.177	tribromomethane + 2-heptanone	0	0	0		0	
68	8.177	2-heptanone (or methyl-ketone)	X	X	X	X	X	X
69	8.277	1,2-cyclooctanediene + ?	X	X				
70	8.760	decanal			X	X		
71	8.961/10.211	toluene?	X	X				
72	9.427	6-methyl-2-heptanone		X		X	X	
73	9.861	2,3-octanedione + benzaldehyde		S			X	
74	9.911	1,3-octadiene + branched C9 alkane	X	X				
75	10.044	2-octanone	X		X			
76	10.961	3-ethyl-2-methyl-1,3-hexadiene	X	X				
77	11.211	alkane		X				X
78	11.245	2,2-dimethyl-3-hexanone		X				
79	11.278	phenol		X	X		X	
80	11.428	?			X	X	X	
81	11.511	(E)-2-octenal	S	X				
82	11.811	1-phenyl-1-pentanone(C11H14O isomer)	X	X	X			X
83	11.828	2-undecanone	X		X	X	X	
84	12.045	nonanal + methylbenzoate	X	X	X	X	X	X
85	12.511	?				X	X	
86	12.695	4-methyl-phenol			X			
87	12.912	dodecane	X	X				X
88	13.245	?(heptanal?+?)		X				
89	13.295	diethylbenzene	X	X				
90	13.528	?	S	S				
91	13.712	naphthalene					0	
92	13.745	decanal					X	
93	13.778	O-isopropenyltoluene(C10H12 isomer)	S	S				
94	14.495	alkane		X		X		X
95	15.062	2-nonadecanone			X		X	
96	15.462	2-methyl-naphthalene				0	0	
97	16.796	1,2-dimethoxy-4-(2-propenyl)-benzene	S	S				
98	18.162	1,1'-(1,3-phenylene)bis-ethanone	S	S	X	X	X	
99	18.179	dimethyl phthalate		X				
100	18.396	butylated hydroxytoluene	0					
101	18.546	1,4-dihydro,-1,4-Ethenonaphthalate	X				X	
102	18.829	?				X		
103	20.947	diethyl phthalate		X				X

Table 5.2: The number and percentage of the total compound composition found within each of the three *Panthera* species which are shared with the other two.

	Number of compounds	Number and % of compounds shared with			
		Tiger	Leopard	Tiger and Leopard	Tiger or Leopard
Lion	58	36 (62%)	16 (28%)	13 (22%)	39 (67%)
Tiger	57	Lion	Leopard	Lion and Leopard	Lion or Leopard
		36 (63%)	15 (26%)	13 (23%)	38 (67%)
Leopard	18	Lion	Tiger	Lion and Tiger	Lion or Tiger
		16 (89%)	15 (83%)	13 (72%)	18 (100%)

Figure 5.5: Mean overlap in compound composition of samples within and between the three *Panthera* species. Overlap is defined as the percentage of compounds shared between the individuals or species being compared.



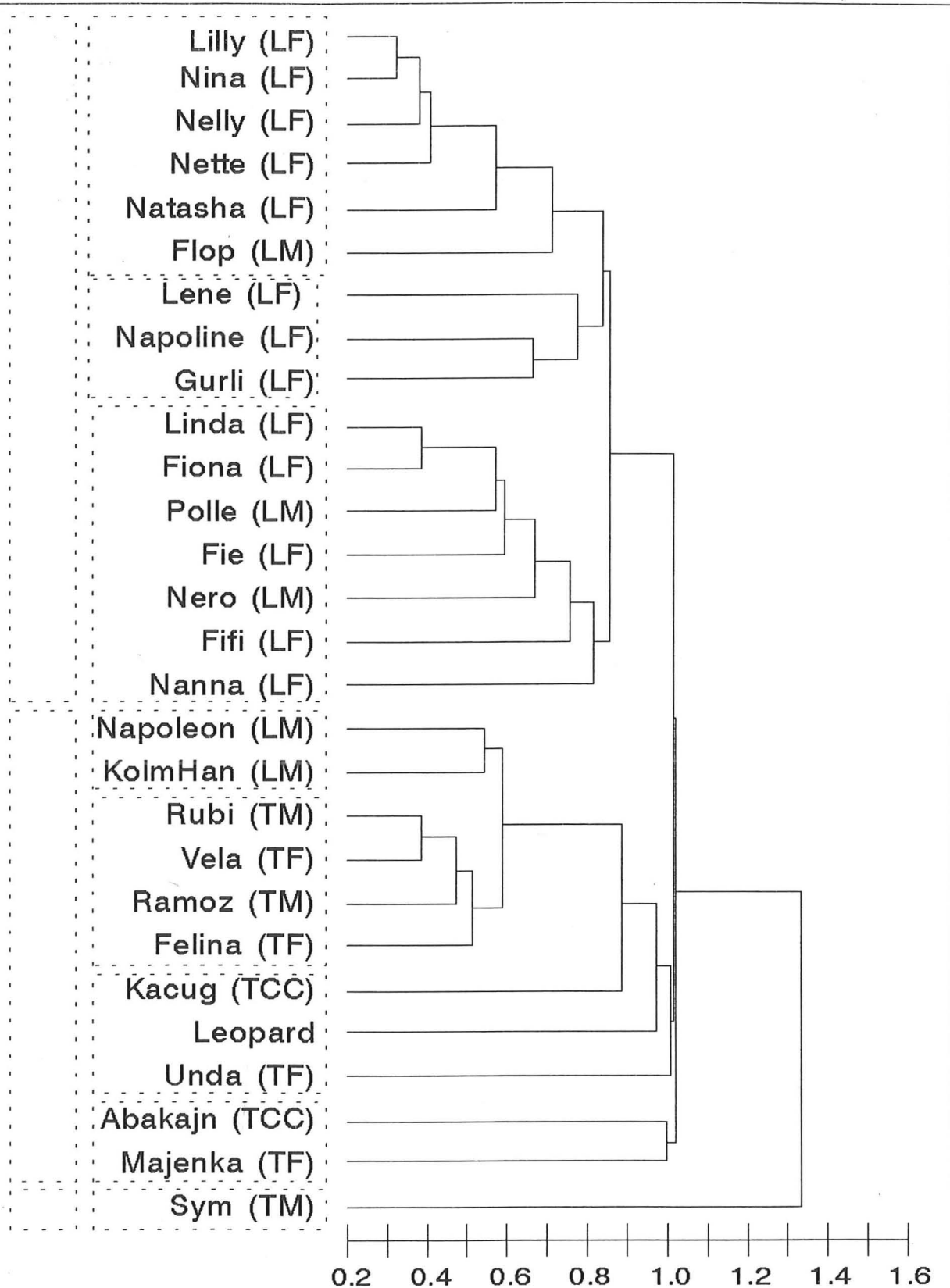


Figure 5.6: Average Linkage Cluster dendrogram showing the "relatedness" between individuals of the three *Panthera* species by the chemical composition of scent marks. The axis at the bottom shows the root-mean-square distance between observations.

Eight smaller clusters merging into three large clusters are present and these are indicated by dotted boxes.

LM: lion male, LF: lion female.

TM: tiger male, TF: tiger female, TCC: tiger cub castrate.

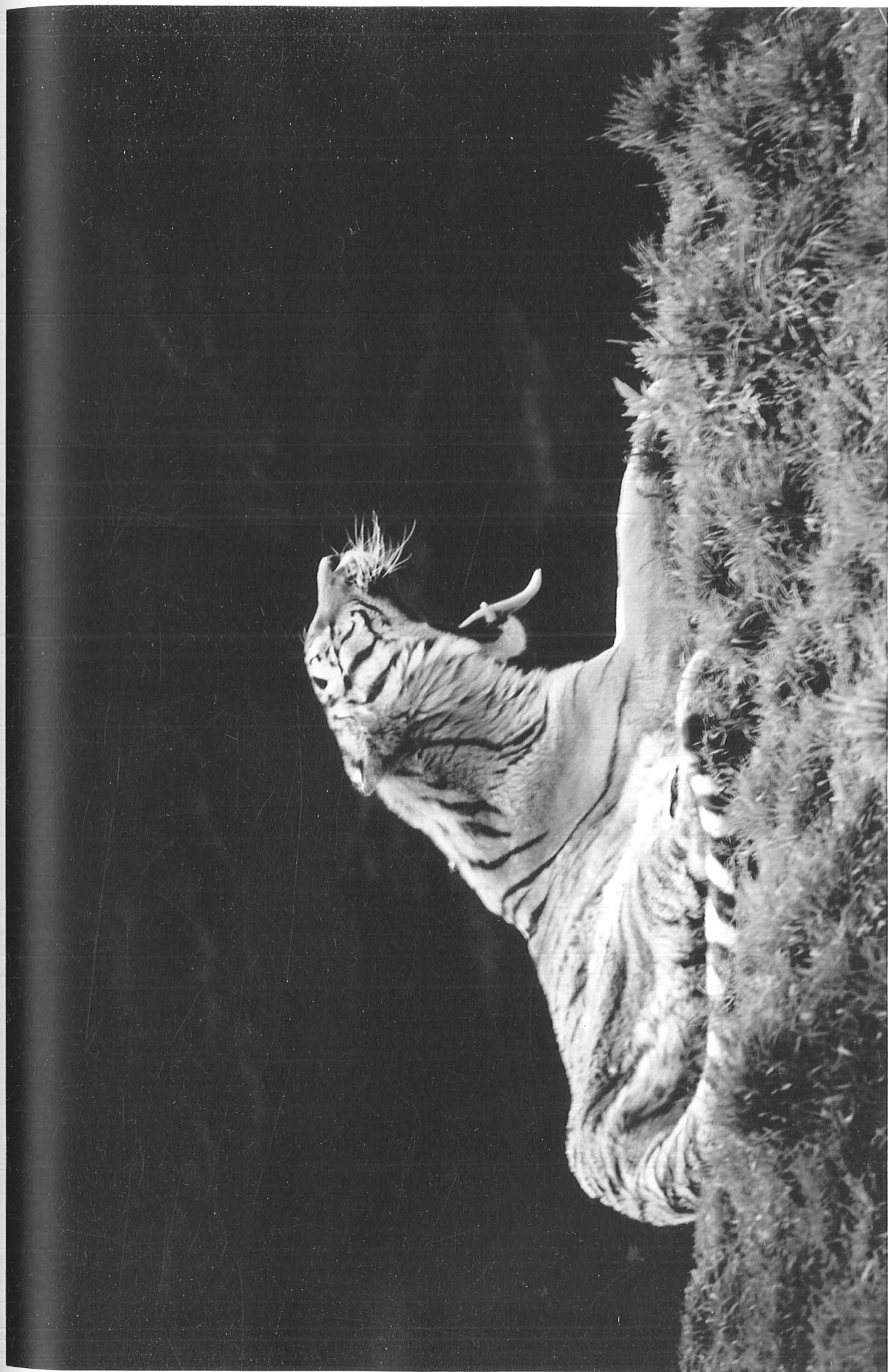
Table 5.3: Compounds which were found in urine samples from lion^a, tiger^a, leopard^a, wolf^b and house mouse^c. An asterisk after a "+" indicates that the compound was also found in control samples in this study.

Name	No.	Lion	Tiger	Leopard	Wolf	Mouse
ethanol	5	+	+	+	+	-
acetone	7	+	+	+	+	+
methylene chloride	9	+	+	+	+	-
benzene	18	+	+	+	-	+
2-pentanone	21	+	+	-	+	-
dimethyl disulfide	37	+	+	-	-	+
toluene	39	+	+	+	+	+
2-heptanone	43	+	+	-	+	+
heptanal	44	+	+	-	+	-
3-hexanone	45	+	+	+	+	-
cyclohexanone	47	+	+	+	+	-
hexanal	49	+	+	-	+	-
benzaldehyde	52	+	+	+	+	+
p-xylene	57	+	+	+	-	+
4-heptanone	61	+	+	+	+	-
styrene	65	+	+	+	+	-
2,4-dithiapentane	66	-	+	-	+	-
phenol	79	+	+	-	+	+
diethyl phthalate	103	+	-	+	+	-

a: results from this study.

b: data from Raymer *et al.* (1986).

c: data from Schwende *et al.* (1986)



CHAPTER 6: CONCLUDING DISCUSSION.

The basis for the present research project and thesis was the formulation of a number of hypotheses which could predict various aspects of the behaviour and chemical composition of scent marks in the three focal *Panthera* species. Behavioural observations and chemical analyses were planned to provide data which could test the validity of the hypotheses, and these results have been presented in the previous three chapters. The time has now come to re-examine the total accumulated evidence and discuss the individual hypothesis in this light. Suggestions for future research will be given where applicable in relation to each hypothesis.

Discussion of hypotheses

Hypothesis 1: The scent marking rates of animals holding a larger territory must be higher than an animal holding a small territory if the density of marks are to be equal.

Prediction: Males generally hold larger territories than females and should therefore have higher rates of marking than them.

Intraspecific evidence.

Lions: Lion males were shown to have higher rates of both *Spraymarking* and *Scrape/urination* than females. They also had a higher percentage of *Spraymarking* in their marking repertoire than females whereas their percentage of *Scrape/urination* was equal to that of the females.

Tigers: Male and female tigers had rates of both *Spraymarking* and *Scrape/urination* which were not significantly different from each other.

Leopards: The male had higher rates of both *Spraymarking* and *Scrape/urination* than the female.

Interspecific evidence.

The lowest rates of *Spraymarking* were found for the leopard female and the lion females, whereas the rate for the tiger females was among the highest, together with the tiger male, the leopard male and the lion males. For *Scrape/urination* the two leopards had the highest rates. The tiger male had a very low rate of *Scrape/urination*.

Validity of Hypothesis 1.

For all three species males generally hold larger territories than the females. In two of the three species, lion and leopard, the males generally also had higher rates of scent marking than the females, whereas the rates for tiger males and females were of roughly the same level. It thus appears that for only two of the three species does the predictions of the hypothesis hold. However, it must be remembered that for leopards the results are based on only one male and one female and therefore these results are only indicative.

There are some further issues which need to be discussed in connection with Hypothesis 1.

One question is whether the scent marking rate of an individual or sex group is inherent or if it is modified according to the area which needs to be marked. If it is inherent then an animal in a small area will have a higher remarking frequency of each marking post than an animal living in a larger area with many more marking posts, but if it is adjustable, then the two animals may have the same frequency of remarking resulting in a higher overall marking rate for the animal in the large area. It was my distinct impression that the animals in the small enclosures had a higher remarking rate of individual scent posts than the animals in the larger enclosures suggesting that the scent marking rate has an element of inherence, but as this was not quantified, this issue will have to await further observations. This issue could also be reflected in the marking rates

of wild population where the territory size of different populations vary considerably depending on habitat and prey density.

A further question which might influence the predictive power of Hypothesis 1 is the fact that the resource on which scent marking is based, namely urine, is a limited resource. This means that the rate of scent marking can be increased only up to a certain level after which the animal will have no more urine left to scent mark with. Therefore if even a relatively small territory is sufficient for the animal to reach this upper limit, and increase in territory size could not lead to a corresponding increase in marking rate. The maximum marking level will depend on the intake of water and general evaporation from the animal and therefore on the habitat which the animal inhabits. It could be that animals with low access to water are able to consciously diminish the amount of urine they deposit with each scent mark, and thus are able to economise their resources and maintain a high frequency of smaller and less powerful scent marks. Animals with ample access to water can afford to be more generous in the amount of urine deposited with each scent mark and will therefore leave bigger and more powerful marks. There is also the possibility that marks from such animals could be more dilute than those from animals with less access to water.

The difference in marking rate observed between the lion sex groups could also be caused by the fact that the males are usually live in small groups or singly whereas the females generally are in larger groups. To obtain an equal density of marks across their territories the females could manage with lower individual marking rates than the males. This point will be discussed more fully in Hypothesis 2.

Further research opportunities in scent marking behaviour.

Qualitative data on scent marking of wild populations of the *Panthera* species are available, but no quantitative data have been published. To obtain this type of information must be the next step in the investigation of scent marking behaviour of these species. This could cast further light on the differences in marking rates between the sexes and between the holders of territories of different sizes. Lions would be the easiest species to observe in the wild as it live in relatively open country, whereas the other four *Panthera* species all

inhabit habitats with dense vegetation or which are difficult to access. However, even lions would pose some problems since a major part of their active period is at night, and it would be difficult to make detailed observations of scent deposition and reactions to scent marks in the dark.

How do the animals react to unmanipulated scent marks deposited by their conspecifics in their natural environment? As there will presumably often be a great lapse in time from the deposition of a mark until it is investigated, this study would be methodologically challenging. One possible though quite costly way of obtaining this type of data would be to use video surveillance fitted with a motion detector overlooking identified scent posts.

Does territorial holders scent mark in a way different from that of nomadic individuals? This was indicated for lions in the present study in that territorial males seem to mark predominantly with *Spraymarking* whereas in the wild it has been found that intruders mostly use *Scrape/urination* (P. Stander pers. comm.) This point deserves further elucidation.

Hypothesis 2: Individuals living in a group-defended territory should have a lower marking rate than those holding their own exclusive territory, if the size of the territories are comparable.

Prediction: Female tiger and leopards will have higher marking rates than female lions because in these two species the females defend a territory alone whereas lions defend group territories.

Evidence.

Only female tigers were found to have higher rates of *Spraymarking* than female lions, whereas the female leopard actually had a lower rate. No major differences were found in the rate of *Scrape/urination* between the females.

Validity of Hypothesis 2.

On the basis of the results presented in this thesis only partial support was found for Hypothesis 2. The leopard female had a lower rate of *Spraymarking* than the lion females, whereas the hypothesis predicts the reverse. One is tempted to suggest that, since the leopard result is based on only one individual, this individual had an abnormally low rate of scent marking, and if more female leopards were observed their true and higher rate of scent marking would be revealed. The fact that she was housed together with a male leopard may have had an influence on her scent marking behaviour. Though this may indeed be the case, it is too early to base any conclusion on these assumptions. Therefore Hypothesis 2 can only be said to hold for lion and tiger females, whereas different mechanisms might operate for the leopard females.

The difference in marking rate found between the lion males and females could have some basis in Hypothesis 2 as well. In addition to their larger territories lion males live either in small groups or singly whereas the females usually live in much larger groups. This leaves the possibility that a combination of large territory and group effect could account for the observed difference, instead of one of these factors being the exclusive reason.

Future study possibilities on group territoriality.

Do female lions mark as a group or individually? As Richardson (1991) reported for the aardwolf, the marking rate of the individual may decrease if a territory is shared with other individuals of the same sex. This could be equally true for female lions, and indeed also in situations where a coalition of male lions hold a territory.

Hypothesis 3: The rates of scent marking of castrated males will be lower than those of intact males.

Prediction: The marking rates of the castrated male tigers will be lower than those of the intact male tiger.

Evidence.

The intact male tiger had a significantly higher rate of *Spraymarking* than the castrates. The marking rate of the male Amur was reduced after his castration.

Validity of Hypothesis 3.

From the evidence presented it does indeed seem that Hypothesis 3 holds. One slight reservation, however, is that the results of the intact male category was based on only one individual, but it is doubtful that the inclusion of more intact males would change the result significantly in the direction of the castrates.

Future research possibilities related to castration.

As the control group in the present study comprised only one individual it would be desirable to enlarge the sample size of this group by observing more captive groups. If castrated individuals of the other *Panthera* species are available somewhere, the conclusions could be extended to include these species also.

Scent samples from castrates should be obtained, if possible, and subjected to a thorough chemical analysis to look for differences in composition or concentration of the constituents in relation to samples from intact males.

Hypothesis 4: An individual of any of the three species will be able to distinguish between the scents of two other individuals of the same species.

Prediction: There will be a measurable difference in the reaction of an animal to scent marks from different individuals.

Evidence.

Data on which to evaluate this hypothesis were collected only for lions and tigers. Both species showed a clear habituation response when presented to four scent marks, three of which were from the same source and one from a different animal. The Odd mark was investigated at a level comparable to the first of the identical marks, whereas the last two of the identical marks were investigated with diminishing interest.

Validity of Hypothesis 4.

Without much doubt it can be said that Hypothesis 4 holds. For both species there was a very clear response in agreement with the prediction of the hypothesis. Indeed, if this ability to discriminate between two scent marks were not present it would be difficult to imagine a way in which scent marks could play a role in communication at all. It certainly allows scent communication to function in a much more sophisticated way than if this ability had been absent. There is no reason for assuming that leopards will be any different from lion and tigers in this respect, but the relevant experiments need to be carried out before it can be said with certainty.

Hypothesis 5: The ability to discriminate between two scent marks of different origin is based on differences in the chemical composition of the scent marks.

Prediction: A chemical analysis of the scent marks should reveal differences in the composition of individual scent marks either at a qualitative or a quantitative level or a combination of these.

Intraspecific evidence.

Lions: A number of compounds were found in only one of the sexes of this species, though none of them was common to all samples from either sex.

The majority of lions had a higher overlap between their own samples than they had with samples from other individuals of the same sex group, and six out of 13 significantly so. Furthermore lion males overlapped significantly more with samples from other males than they did with females, whereas the females did not overlap more within their own sex than they did with male samples.

Male lions had a significantly higher absolute concentration of 2-butanone than females, and females had a significantly higher relative concentration of acetone than did males.

Tigers: Some compounds were found in samples from only male or female tigers, but none of these compounds was present in all samples from either sex.

All tigers overlapped more within their own samples than they did with other samples from their own sex group but only one significantly so. Males did not overlap significantly more with other males than they did with females, and the same was true for "female with female" and "female with male" overlap.

There was no difference in the concentration of any of the compounds found in tiger samples from intact males and females. The castrated male cubs had a significantly higher absolute and relative concentration of 5-hydroxy-4-methyl-6-hepten-3-one than the intact males.

Leopards: The leopard samples analysed revealed no differences in compound composition or concentration between the sexes.

Interspecific evidence.

Eight compounds were common to the urine samples of all three species. Of the compounds found in lion and tiger urine respectively 33% were specific to each of these species, whereas the leopard shared all its compounds with either lion or tiger samples.

Samples from lions overlapped significantly more with other lion samples than they did with either tiger samples or leopard samples. Tiger samples also had a significantly higher

"within species" overlap than "between species" overlap. Leopard samples overlapped equally with lion and tiger samples.

Validity of Hypothesis 5.

Twice as many lion samples as of tiger samples were analysed, and twice as many tiger samples as leopard samples were analysed. Therefore the lion results are the most reliable. These results show that there are indeed chemical differences between individuals and sexes on both a qualitative and a quantitative level as was predicted by the hypothesis. Similar but less pronounced differences were found for tiger samples, whereas the leopard samples provided no real evidence either for or against the hypothesis.

The differences in compound composition and concentration found in this study are probably not the only ones which exist. The method of analysis used was not optimal for all classes of compounds, so it is likely that once additional analyses have been performed more compounds will be identified and this will lead to the discovery of more differences in compound composition and concentration between individual, sexes and species. There are no reasons to assume that such differences are not also present in tiger and leopard samples.

For a couple of other species it has been reported that castration leads to a fall in the number and concentration of compounds present in the urine (wolf: Raymer *et al.* 1984, 1986; mouse: Schwende *et al.* 1986). No samples could be collected for the adult castrates, but samples were analysed for the big tiger cubs which had also been castrated. There were no immediate evidence for a smaller number of compounds or a fall in concentration level between these samples and those from the intact males. Whether further analysis will reveal such differences or whether tigers (and the other *Panthera* species?) are different in this respect from wolves and mice remains to be seen.

Future research related to chemical composition.

The chemical data presented in this thesis, together with the other studies which have been reviewed, start to provide a comprehensive picture of the chemical composition of *Panthera* scent marks. However each of these studies has tested different fractions of the scent marks using different techniques. Therefore a single study analysing all fractions of the scent marks with appropriate techniques would be desirable. If several samples from a number of individuals are analysed it would also give a good idea of the variation in chemical composition at both the individual as well as the species level.

By using the method for urine collection developed in this study, scent marks could be collected from wild animals and their chemical composition established and compared to that from captive animals. This could provide answers to the questions of the effect of diet and possible geographic differences.

Hypothesis 6: Animals are able to distinguish between scent marks from males and females.

Prediction: A chemical foundation for this distinction must be present. When investigating a scent mark the reaction or intensity of investigation will vary depending on the sex of the depositing animal and the investigating animal.

Evidence.

The chemical foundation for the prediction was discussed in relation to Hypothesis 4.

Males of both lion and tiger showed greater interest (i.e. more *Scenting* and *Flehmen*) in marks from males than marks from females. No major differences were found for the females of these two species when investigating these mark types. The leopard male showed a tendency similar to that of male lions and tigers though not quite as pronounced.

Validity of Hypothesis 6.

For both the male and the female sex group of all three species there is a intra-sex competition for territories and therefore one would expect the scent marks of other individuals within the same sex group to be of particular interest as they would tell of possible trespassers or territorial holders. From the evidence presented in this thesis this does indeed appear to be the case for the male sex group. Females on the other hand showed no particular interest in marks from other females over the other categories of experimental marks and this is somewhat puzzling. No explanation for this result has been found.

Future research related to experimental scent presentations.

By using appropriate chromatographic techniques the different fractions of a scent mark can be separated and collected. Assuming they can be collected in sufficient amounts they can subsequently be presented to test animals in order to evaluate the biological importance of each of the fractions. Alternatively or as a supplement synthetic equivalents of some of the naturally occurring compounds can be used when performing scent mark presentations to the extent they are commercially available or can be produced in the laboratory.

Hypothesis 7: Males of the three species should be able to distinguish scent marks from females in oestrous apart from those of non-oestrous females.

Prediction: There will be a measurable difference in the reaction of males to these two categories of scent marks.

Evidence.

The lion males and the intact tiger male showed more *Scenting* and *Flehmen* towards

this category than towards non-oestrous female marks. As no marks were obtained from oestrous female leopards the leopard male could not be tested in a similar fashion.

Validity of Hypothesis 7.

According to the evidence presented in this thesis males of at least two *Panthera* species, lion and tiger, appear to be able to distinguish marks from oestrous females from those of non-oestrous females. That this ability exists makes good biological sense. If males can recognise the presence of an oestrous female within their territory from her scent marks he will have a greater chance of finding and mating with this female.

There are no reasons to assume that leopards will be any different from lions and tigers with regard to this ability to distinguish marks from oestrous females, but the actual proof of this will have to await further experiments.

Hypothesis 8: The animals are able to distinguish between marks from another individual of its own species and that of a related species.

Prediction: The reaction of the animals to marks from its own species and those of the other *Panthera* species should be measurably different.

Evidence.

Lions: The lions generally showed a high interest in the scent marks from the two foreign species, tiger and leopard. Tiger marks received more *Scenting* and leopard marks more *Flehmen* than the other mark types.

Tigers: The intact tiger male and the females showed less interest in lion marks than tiger male and tiger female oestrous marks. The castrated adult males showed much more interest in lion marks than tiger male marks.

Leopards: Only the male leopard showed higher interest in lion and tiger marks than in marks from leopards.

Validity of Hypothesis 8.

In all three species a difference in reaction was seen between the investigation of "same species marks" and "other species marks" for most of the sex groups. Sometimes the reaction was higher towards the "other species" marks and sometimes it was lower, but the fact that a difference was present indicates that the animals are able to distinguish between species and non-species marks. Thus, considerable evidence in favour of Hypothesis 8 was found.

Scent marking in territorial behaviour.

In the previous chapters it has been demonstrated that the urine of at least two of the three *Panthera* species studied is chemically well adapted for communicatory purposes. It has also been shown that an individual is able to discriminate between the scent marks of two foreign animals. The question then arises of how scent marks are employed by the animals. If scent marks are to play a role in territoriality at all, it is a prerequisite that an animal is able to tell by the scent environment that it is moving in foreign territory, i.e. that it is able to discriminate between its own scent and that of another animal (discrimination of the first order). In order to distinguish between different territories, an animal would have to be able to distinguish between the scent from two foreign individuals (discrimination of the second order). The ability to recognise another individual by its scent could also be of value in certain situations (discrimination of the third order), e.g. if an animal faces the decision of whether or not to challenge a particular territory holder, it may be helped in this decision if it has previous experience of conflict with the same individual.

Although the basic requirements for Gosling's "scent-matching" hypothesis (Gosling 1982) appear to be present in the *Panthera* species (discrimination of the second order), it

seems very unlikely that it is applicable to these animals. In contrast to evidence found for a number of ungulate species, no signs of a scent-matching ritual has been found for any of the larger carnivores. Intruders are most often attacked without any questions being asked first. Indeed this limitation to the applicability of his hypothesis was pointed out by Gosling himself. This leaves the possibilities of scent marking working either by deterring and intimidating a potential intruder or by increasing the confidence and orientation of the resident animal(s). A combination of these factors is also possible. The basic requirement for these mechanisms to work is only that an animal should be able to distinguish its own scent from that of other individuals, i.e. first order discrimination, but it would be able to work with much more subtlety if second and third order discrimination were also present.

There are three different classes of potential territory invaders. The first comprises adults of the same sex as the territorial holder, the second adults of opposite sex to that of the territory holder, and the third dispersing subadults who are on the lookout for areas in which to establish their own territories. The first group can be split up into two subgroups; neighbouring territory holders and nomads, which for various reasons do not hold territories of their own.

The neighbouring territorial holders are likely to be familiar with each other through regular visual, auditory and olfactory contact. Between such individuals scent marks are likely to signal a *status quo* in the territorial ownership, i.e. there is very little possibility of extending your territory until a change in the scent mark pattern or identity indicates that the neighbouring territory has been vacated or taken over by a new male. Nomads are always travelling in unfamiliar country, and from the scent marks of the resident males they would be able to extract information about their identities and whereabouts. In this way they could move around within foreign territories with a minimal risk of encountering a resident male. There is evidence that nomads also deposit scent marks when inside foreign territories. Why they should do this is not quite clear as it tells the resident male that somebody is now trespassing on his territory. One hypothesis which could explain this is that the territorial holder and the intruder use different types of scent marking. This was indicated in the findings of the present study, in that territorial males, at least in lions, seem to use *Spraymarking* rather than *Scrape/urination* in territorial contexts. It is further supported by the observations of Dr Philip Stander who during his many years of fieldwork on lions in Namibia, came to the conviction that territorial males use

Spraymarking rather than *Scrap/urination* whereas intruders show the opposite pattern (P. Stander pers. comm.). This suggests that the main part of the territorial message is conveyed by *Spraymarking* whereas *Scrape/urination* may be less important in this context. If therefore intruders marked predominantly by *Scrape/urination* rather than *Spraymarking*, it may convey the message to the resident male that somebody is trespassing on his territory temporarily, but that the invader does not make any territorial claims. In this way the resident male might feel less threatened by the intruder and hence be less aggressive towards it. In this way scent marking could be beneficial for the intruder as well.

Intruders of the opposite sex are unlikely to provoke a territorial response from residents of either sex. Male territories frequently overlap several female territories without apparent conflicts. The only situation in which an intruder could pose a threat is when a female with young is faced with a male intruder. Several examples from lions, tigers and leopards are known in which nomadic males have killed the offspring of the resident female (Bertram 1975; Packer and Pusey 1984; Bailey 1993). In this case scent marks could help both the female and the intruder to keep track of each other and thereby avoid conflict.

The final class of territorial invader consists of the dispersing subadults who have been evicted from their natal territories. For lions these will primarily be males, as females tend to stay in their natal pride when reaching sexual maturity, whereas for tigers and leopards both males and females disperse to establish themselves as independent territory holders (Hanby and Bygott 1987; Hanby *et al.* 1995). Scent marking is potentially a very effective way for a resident animal to tell the dispersing subadults that the area in question is already occupied by a mature animal and that they had therefore better move on. For the subadults, scent mark information could be lifesaving in that by avoiding areas with scent marks they avoid exposing themselves to the risk of attack by a mature animal with much better fighting abilities. By tuning in to the scent messages they would be able to find an area, probably of low resource value, where they can live uncontested until they are strong enough to challenge other territorial holders with territories of a higher resource value.

The economics involved in marking a territory with scent has been a point of great discussion. Some species seem to mark the periphery of their territory with greater intensity than the interior parts, whereas others seem to have the highest density of scent

marks somewhere between the centre and borders of the territory. The actual physical size of the territory will have a great influence on this matter as an increase in territory size will make it more difficult and uneconomic for an animal to keep up a high density of scent marks along the border.

The type of mark which an animal leaves will influence the maximum frequency and density of marks which is possible for that animal. If marks are physical impressions left on the environment in the form of clawing of trees or scrapes on the ground then a very high frequency of marking can be kept up resulting in an overall high density. If, on the other hand, excretions or secretions are left as marks either alone or in combination with physical impressions then each mark will cost the animal a reduction in the excretory or secretory resource used for the mark leading to an eventual depletion of this resource if it is not renewed. The renewal of the resource is dependent on the animal having access to precursors of the resource in the form of solids (food) or fluids (water) or both. The timing of the access to and conversion of such precursors into new marking material may well lead to a temporary inability of an animal to leave effective scent marks thus lowering the "security level" in the territory. This problem of resource depletion would face the holders of large territories requiring a high marking frequency more often than holders of smaller territories. One way to alleviate the problem could be by changing the pattern of scent marking from a primarily peripheral marking pattern to a more interior or "core area" oriented one.

The territories of the three *Panthera* species studied here vary greatly from place to place. In rich habitats of high prey availability such as the Ngorongoro Crater for lions, territories are small whereas more marginal and harsh habitats with an unpredictable prey availability leads to bigger territories as is seen in the Siberian tiger. The smaller territories seem to be much more eagerly defended than the larger ones. This has been documented for lions by McComb *et al.* (1993, 1994), Heinsohn and Packer (1995) and Heinsohn *et al.* (1996) in a series of playback experiments where the Ngorongoro prides approached suspected intruders much more readily, even when the odds of winning were low because of the numbers of intruders, than their Serengeti counterparts.

Based on these speculations one would expect the Ngorongoro lions to concentrate their scent marking effort along the border to erect an impenetrable "shield" around their territory, whereas the lions in less prey rich areas might well exhibit the more interior

oriented marking pattern. The same difference in scent marking strategy is probably also reflected for the other two species where differences in territory size occurs.

A point of interest with regard to the lipid content of urine and its relation to territorial behaviour needs mentioning. If the lipid concentration in urine is correlated with the kidney fat index, as was suggested by Hewer *et al.* (1948), it would mean that the scent marks deposited by strong, fit and well-fed animals would contain more lipids, and hence be of much greater longevity, than those deposited by under-nourished individuals. In this way a fit animal could advertise its presence in a larger area than an unfit animal, and therefore hold a bigger territory, and at the same time it would demonstrate that it is a fit animal.

Concluding remarks.

During the work on this project progress has been made in understanding several aspects of the marking behaviour of the three *Panthera* species, as well as on the chemical composition of their scent marks. However, as was pointed out in the previous sections of this chapter, numerous important questions are still unanswered, and much more work need to be done within this field. It is hoped that the work described in this thesis, and in the publications resulting therefrom, will whet the appetite of other researchers.

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APPENDIX 1.

The chemical compounds identified in urine samples from the three *Panthera* species studied are listed indicating their Retention time, Name and Major peaks from their mass spectrums.

Number	Retention time	Name	Major peaks
1	1.126	trimethylamine	58, 59, 42, 30, 43, 57
2	1.342	pentane?	43, 42, 20, 29
3	1.376	pentane	43-41, 27, 29, 57, 72
4	1.409	?	45, 27, 29, 30, 72
5	1.426	ethanol	31, 45, 27, 43, 46
6	1.592	azetidine?	29, 27, 57, 26, 39
7	1.642	acetone	43, 58, 42
8	1.742	carbon disulfide	76, 78, 44, 32, 38
9	1.909	methylene chloride	49, 84, 86, 51, 47, 88
10	2.093	methylmethylether	73, 57, 43, 29
11	2.292	hexane	57, 41, 43, 44, 56, 86, 76
12	2.442	diisopropyl ether	45, 43, 87, 41, 59, 69
13	2.676/2.809	2-butanone	43, 72, 29, 57, 44
14	2.809	?	75, 43, 45, 47, 72, 76
15	2.993	(+/-)-2-butanol	45, 59, 41, 31, 43
16	3.026	tetrahydrofuran (THF) + chloroform	83, 42, 85, 41, 71, 72, 47
17	3.243/3.610	3-methylbutanal	41, 44, 58, 39, 29, 57, 71
18	3.493	benzene + column bleeding	78, 147, 77, 52-50
19	3.560	acetic acid + trichloroethylene	43, 45, 60, 95
20	3.593	2,2,3,3-tetramethylbutane	57, 56, 41, 43, 29-27, 99
21	3.693/4.293	2-pentanone	43, 86, 57, 58, 41, 27, 29, 71, 90
22	3.693/3.776/4.360	3-methyl-1-butylamine	30, 41, 70, 55, 87
23	3.693/4.360/4.393	pentanal	44, 29, 41, 57, 58, 43
24	3.709/4.393	amine?	44, 57, 41, 86, 58, 29
25	3.726	2-methyl-butanol	29, 57, 41, 58, 27, 55, 86
26	3.776	heptane	43, 41, 57, 71, 56, 70, 100
27	3.793	?	43, 41, 57, 29, 86
28	4.093	trichloroethylene	130, 95, 132, 97, 60, 134
29	4.260	s-methyl ester ethanethioic acid + 2-pentanone	43, 90, 86, 45, 59, 42, 47
30	4.276	2-pentene	55, 42, 41, 43, 70, 31, 57
31	4.376	n-ethyl-1-propylamine	30, 44, 41, 58, 70, 87
32	4.443	5-hydroxy-4-methyl-6-hepten-3-one	57, 29, 86, 76, 56
33	4.460/5.893	1-methoxyethanethiol	77, 45, 78
34	4.576	bromodichloro-methane	83, 85, 47-50, 129, 127
35	4.610	1-pentene	42, 55, 41, 31, 29, 70, 57
36	4.843	hexanal	44, 56, 41, 57, 29, 43, 72, 82
37	5.076	dimethyl-disulfide	94, 79, 45-48
38	5.393	3-methyl-1-butanol	55, 42, 70, 41, 43, 57
39	5.410	toluene	91, 92, 69, 41
40	5.493	3-methyl-2-pentanone	43, 41, 57, 72, 100, 39, 56
41	5.593	octane	43, 41, 57, 85, 71, 56, 29
42	5.743	3-methyl-pentane	57, 56, 41, 43, 29, 72
43	5.860	2-heptanone	43, 58, 71, 59, 85, 99, 114
44	5.926/8.277	heptanal	41, 44, 70, 43, 55, 57, 81, 86, 96
45	6.043	3-hexanone	43, 57, 29, 27, 71, 100
46	6.043	tetrachloroethylene	166, 164, 129, 131, 168, 94, 96
47	6.110	cyclohexanone	55, 42, 98, 41, 39, 69, 70, 83
48	6.343	dibromochloromethane + hexanal	129, 127, 131, 44, 56

Number	Retention time	Name	Major peaks
49	6.343	hexanal (with contam.)	44, 56, 41, 57, 43, 29, 72, 82
50	6.477	?	133, 151, 135
51	6.610	2-pentylfuran	81, 82, 53, 138, 95
52	6.777/9.777	benzaldehyde	105, 106, 77, 51, 50, 78, 107
53	6.943/10.194/10.228	octanal	41, 43, 29, 57, 56, 44, 84, 69, 100
54	7.094	chlorobenzene	112, 77, 114, 51, 50
55	7.260	4-methyl-3-penten-2-one	55, 98, 43, 83, 39
56	7.277	ethylbenzene	91, 51, 77-79, 92
57	7.427	p-xylene	91, 106, 105, 77, 51
58	7.527	nonane	43, 57, 41, 85, 56, 71, 29
59	7.627	1-octanal	41, 55, 56, 43, 69, 70, 83, 84, 31
60	7.644	amine?	43, 114, 41, 72, 55, 129, 59, 45
61	7.727/7.760	4-heptanone	43, 71, 27, 61, 41
62	7.744	2-n-butyl-furan	81, 124, 82, 53, 114
63	7.894/11.995/12.045	nonanal	41, 57, 29, 43, 56, 70-67, 82, 98
64	7.927	3-methyl-1-pentanol	56, 43, 55, 41, 42, 69, 31, 29, 27
65	7.960	o-xylene + styrene	104, 103, 78, 106, 51, 77
66	7.994	2,4-dithiapentane	61, 108, 45-47, 35, 110
67	8.177	tribromomethane + 2-heptanone	173, 171, 175, 81, 91, 93, 252
68	8.177	2-heptanone (or methyl-ketone)	43, 58, 71, 59, 41
69	8.277	1,2-cyclooctanediene + ?	108, 93, 91, 95, 29, 39, 41, 67, 81
70	8.760	decanal	41, 57, 43, 55, 29, 70, 82, 95, 112
71	8.961/10.211	toluene?	91, 92, 132
72	9.427	6-methyl-2-heptanone	43, 58, 41, 71, 110
73	9.861	2,3-octanedione + benzaldehyde	43, 99, 71, 105, 106, 91
74	9.911	1,3-octadiene + branched C9 alkane	57, 43, 54, 67, 41, 81, 110
75	10.044	2-octanone	43, 58, 41, 59
76	10.961	3-ethyl-2-methyl-1,3-hexadiene	67, 95, 124, 39, 41
77	11.211	alkane	57, 43, 71, 41, 85, 55
78	11.245	2,2-dimethyl-3-hexanone	57, 71, 41, 43, 56, 85
79	11.278	phenol	94, 66, 65, 39, 55, 95
80	11.428	?	43, 114, 41, 55, 72, 29, 129
81	11.511	(E)-2-octenal	41, 55, 70, 39, 83, 57
82	11.811	1-phenyl-1-pentanone(C11H14O isomer)	105, 77, 120, 51, 43
83	11.828	2-undecanone	58, 43, 71, 59, 57, 41, 27
84	12.045	nonanal + methylbenzoate	73, 57, 41, 43, 267, 55, 29
85	12.511	?	99, 57, 155, 41, 139, 112
86	12.695	4-methyl-phenol	107, 108, 77, 79, 51, 53
87	12.912	dodecane	57, 43, 71, 41, 85, 29
88	13.245	?(heptanal?+?)	41, 55, 39, 83, 70, 57, 29, 43
89	13.295	diethylbenzene	119, 91, 134, 107, 120, 117
90	13.528	?	95, 93, 41, 110, 67, 79, 55
91	13.712	naphthalene	128, 129, 127, 81, 70
92	13.745	decanal	41, 43, 57, 55, 70, 82, 95, 112
93	13.778	O-isopropenyltoluene(C10H12 isomer)	117, 132, 115, 91, 131, 65
94	14.495	alkane	43, 57, 71, 41, 85, 29, 55, 70
95	15.062	2-nonadecanone	58, 43, 59, 27
96	15.462	2-methyl-naphthalene	142, 141, 115, 139, 143
97	16.796	1,2-dimethoxy-4-(2-propenyl)-benzene	178, 91, 107, 147, 163, 103
98	18.162	1,1'-(1,3-phenylene)bis-ethanone	147, 91, 43, 119, 162
99	18.179	dimethyl phthalate	163, 77, 76, 50
100	18.396	butylated hydroxytoluene	205, 220, 206, 57
101	18.546	1,4-dihydro,-1,4-Ethenonaphthalate	153, 154, 152, 151, 155, 76
102	18.829	?	41, 99, 39, 56, 55
103	20.947	diethyl phthalate	149, 177, 76, 150

Appendix 2

Methods for Statistical Analysis of Scent Mark Experiments

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Introduction

When a scent mark is presented to a lion, two main types of reactions can be observed, Scenting and Flehmen. The frequency of either reaction may depend on the sex and age of the lion, the type of scent mark, and the experimental conditions. The aim of the statistical analyses is to determine some structures in these dependencies.

The experimental design is quite complicated, some experiments were conducted on a single individual, others on groups of different sizes, where the different animals in the group in some cases could be identified and observed individually, in other cases they could not be distinguished individually, such that they could only be recognized as a male, a femae, or a cub. The number of times a scent mark was presented to an individual or a group also varied quite considerably. The number of reactions recorded is rather low in most cases, in particular with regard to Flehmen. These properties of the data make use of standard statistical methods based on the normal distribution inappropriate. A tailor-made method for this type of experimental data had to be developed. The method may be used for similar types of experimental data.

Comparisons will be made of the frequencies of the two types of reactions for each type of experimental set up. For each type of reaction comparisons will be made between the frequencies resulting from the presentation of different scent marks to males, females, and cubs, respectively, in each of the four experimental series. Likewise the frequencies will be compared for males, females, and cubs for each type of scent mark in each of the four experimental series. Finally, comparisons will be made between the frequencies of the two types of reactions in the different experimental series. All statistical tests will be carried out as pairwise comparisons, as this type of comparisons makes it possible to rely on exact methods, which is necessary because of the small number of reactions in most cases.

The same methods will be used for both types of reaction and for comparisons of their respective frequencies. Therefore, when developing the methods we will only speak of reaction, and then only one of the types is considered.

Assumptions and basic probability distributions

Consider first the presentation of one scent mark to a single lion. We will assume that the number of reactions during the actual presentation is Poisson distributed with Poisson parameter λ , which is to be interpreted as *the frequency of the actual type of reaction of a single lion to the scent mark during a single presentation*. This is the basic parameter, which we will compare for the different types of set up. Basically, we will assume that each individual lion has its own specific parameter for the actual type of scent mark under the given experimental conditions.

If the number of reactions during the actual presentation is Poisson distributed with Poisson parameter λ and we call the recorded number of reactions a , then the probability for this result can be written in the following way,

$$\frac{\lambda^a}{a!} e^{-\lambda} \quad \text{where } a = 0, 1, 2, \dots$$

This is the usual way of writing this standard distribution, whose properties are discussed in many textbooks in statistics. If the same lion has been presented to the same type of scent mark m times under the same experimental conditions, the total number x of reactions for the whole series of presentations will be Poisson distributed with parameter $m\lambda$, and the probability is

$$\frac{(m\lambda)^x}{x!} e^{-m\lambda} \quad \text{where } x = 0, 1, 2, \dots$$

The parameter λ has to be interpreted, as mentioned above, as the frequency of the actual type of reactions of a single lion to one presentation of one scent mark. This parameter may vary with sex, age, and other experimental conditions. We will call λ *the basic frequency* for the actual experimental set up.

If we consider a group of n lions which has been presented for the same scent mark under the same experimental conditions m times, the different individuals are not distinguishable. Hence, we have to assume that the basic frequency λ for each individual has to be the same for all individuals in the group. Then if y is the total number of reactions from all the n members of the group during the m presentations, y will be Poisson distributed with parameter $nm\lambda$, the probability will be equal to

$$\frac{(nm\lambda)^y}{y!} e^{-nm\lambda} \quad \text{where } y = 0, 1, 2, \dots$$

Consider now two groups of lions. In the first group there are n_1 individuals and they have been presented for a scent mark under the same conditions m_1 times. Their common basic frequency is λ_1 . In the second group there are n_2 individuals, they have

been presented for a scent mark m_2 times, and their common basic frequency is λ_2 . Let z be the total number of reactions for all individuals in both groups taken together. Then the variable z is Poisson distributed with parameter $(n_1 m_1 \lambda_1 + n_2 m_2 \lambda_2)$ and with probability equal to

$$\frac{(n_1 m_1 \lambda_1 + n_2 m_2 \lambda_2)^z}{z!} e^{-(n_1 m_1 \lambda_1 + n_2 m_2 \lambda_2)} \quad \text{where } z = 0, 1, 2, \dots$$

If we may assume that the basic frequencies $\lambda_1 = \lambda_2$, e.g. that the two groups have been presented for the scent marks under the same conditions, and we denote the common, basic frequency with λ , then z is Poisson distributed with parameter $(n_1 m_1 + n_2 m_2) \cdot \lambda$, and the probability is equal to

$$\frac{[(n_1 m_1 + n_2 m_2) \lambda]^z}{z!} e^{-(n_1 m_1 + n_2 m_2) \lambda} \quad \text{where } z = 0, 1, 2, \dots$$

One or both groups may consist of only a single individual, then either $n_1 = 1$ or/and $n_2 = 1$. These arguments can also be applied if the groups consist of single individuals, which have been presented to the same scent marks as a group, but identified and observed individually.

These results may easily be generalized. If there are k groups of lions with n_i individuals in group i , which has been presented for a scent mark m_i times, and their common basic frequency is λ_i , then the total number of reactions from all individuals in all k groups will be Poisson distributed with parameter

$$n_1 m_1 \lambda_1 + \dots + n_i m_i \lambda_i + \dots + n_k m_k \lambda_k$$

If we can assume that all basic frequencies are equal, i.e. $\lambda_1 = \dots = \lambda_i = \dots = \lambda_k$, and we call the common, basic frequency λ , then the total number of reactions from all individuals in all the groups will be Poisson distributed with parameter

$$(n_1 m_1 + \dots + n_i m_i + \dots + n_k m_k) \lambda$$

We will denote the sum $(n_1 m_1 + \dots + n_i m_i + \dots + n_k m_k) = M$, which is the total number of presentations all individuals in all k groups have received, when we consider the received presentations for each individual and add them together for all individuals within each group and for all groups. If the total number of reactions of the actual type in all these k groups is called t , then t is Poisson distributed with parameter $M \lambda$, and the probability is

$$\frac{(M \lambda)^t}{t!} e^{-M \lambda} \quad \text{where } t = 0, 1, 2, \dots$$

These probability distributions will be used to derive estimates of the basic frequencies for different experimental set ups and to derive statistical tests.

Example 1

In order to illustrate the notation given above, consider the outdoor experiments in Copenhagen with scent mark: "Pl Male". There is a single male, which has received $m = 5$ presentations of this scent mark and has given $y = 15$ Scentings. Thus for Males $M = 5$ and $t = 15$. There are two females, which have been presented to the scent mark 4 times and observed individually. Considered individually, the former one has received $m_1 = 4$ presentations and given $y_1 = 5$ Scentings, the latter one has received $m_2 = 4$ presentations and given $y_2 = 3$ Scentings. As there are two single females $n_1 = n_2 = 1$, and thus we get $M = 8$ and $t = 8$. Finally, there is a group consisting of $n = 4$ cubs, which has got $m = 4$ presentations as a group and given in all $y = 18$ Scentings. For the cubs we get $M = 16$ and $t = 18$.

In the tables presenting the *Observations for the different localities and conditions*, the number of Received Stimuli, i.e. M , is given for all combinations of Males, Females, and Cubs on the one hand and the different scent marks on the other. Furthermore, for the same combinations of categories of lions and scent marks the number of recorded reactions, i.e. t , is given for Scenting and for Flehmen.

Estimation of the basic frequencies

Consider an experimental set up and a group (or a collection of groups) for which we may assume the basic frequency λ to be constant for all individuals. In order to illustrate the notation consider the example above. We have three such collections of groups: Males (one individual), Females (two groups with one individual in each), and Cubs (one group with 4 individuals). The last formula given above will be used to derive the estimate for the basic frequency. This can be calculated in the following way

$$\hat{\lambda} = \frac{t}{M} \quad \text{with standard deviation} \quad \frac{\hat{\lambda}}{\sqrt{M}}$$

Example 2

By means of the data in Example 1 for Males the basic frequency is estimated to

$$\hat{\lambda} = 15/5 = 3.000, \quad \text{for Females the basic frequency is estimated to}$$

$$\hat{\lambda} = 8/8 = 1.000, \quad \text{and for Cubs the basic frequency is estimated to}$$

$$\hat{\lambda} = 18/16 = 1.125.$$

Below the tables of *Observations for the different localities and conditions* the estimated basic frequencies, or frequency parameters, for Scenting and for Flehmen are given for all combinations of categories of lions and types of scent marks. The standard deviations have not been calculated.

Comparisons of basic frequencies for Scenting and Flehmen reactions

The basic frequency λ is likely to be different for the two types of recorded reactions. We want to test the statistical hypothesis that the basic frequencies for Scenting and for Flehmen are equal. As the basic frequency of scenting may be higher than that of Flehmen, but hardly lower, the alternative hypothesis is that the basic frequency of Scenting is higher than that of Flehmen. If we call the basic frequency of Scenting λ_1 for the actual group and that of Flehmen for λ_2 , then the null hypothesis which will be tested is: $H_0: \lambda_1 = \lambda_2$ against the alternative hypothesis $H_A: \lambda_1 > \lambda_2$. This alternative hypothesis is *one-sided* in contrast to the *two-sided* alternative hypothesis discussed in the next section.

As mentioned above, if the number of received presentations is M for a given group of lions, the total number t of reactions of a specified type, either Scenting or Flehmen, will be Poisson distributed with Poisson parameter $M\lambda$. For the same group of individuals the number of received presentations M is the same for both types of reactions.

The test for this null hypothesis is derived in the following way. Let the number of recorded Scentings be t_1 , then t_1 is Poisson distributed with Poisson parameter $M\lambda_1$. The number of recorded Flehmens is t_2 , which is Poisson distributed with parameter $M\lambda_2$. Let s be the total number of recorded reactions, i.e. $s = t_1 + t_2$. Then s is Poisson distributed with Poisson parameter $(M\lambda_1 + M\lambda_2) = M(\lambda_1 + \lambda_2)$. The conditional distribution of the number of recorded Scentings, t_1 , for the first group, given the total number of recorded reactions s , is binomially (Bernoulli) distributed with parameters s and

$$\frac{M\lambda_1}{M(\lambda_1 + \lambda_2)} = \frac{\lambda_1}{\lambda_1 + \lambda_2}$$

where the latter one is called the binomial parameter. The probability for this result is given as

$$\binom{s}{t_1} \left(\frac{\lambda_1}{\lambda_1 + \lambda_2} \right)^{t_1} \left(1 - \frac{\lambda_1}{\lambda_1 + \lambda_2} \right)^{s-t_1} \quad \text{where } t_1 = 0, 1, \dots, s$$

The first factor in this formula is the binomial coefficient. If the null hypothesis H_0 holds, i.e. that the basic frequencies for Scenting and Flehmen are equal, then the binomial parameter will be equal to $\frac{1}{2}$, if the alternative hypothesis is correct, then the binomial parameter will be larger than $\frac{1}{2}$. The binomial distribution and its properties and the type of conditioning carried out here may be found in many statistical text books. Thus with the conditional distribution given above the original H_0 and H_A are transformed into the following null hypothesis and alternative hypothesis, respectively:

$$H_0: \frac{\lambda_1}{\lambda_1 + \lambda_2} = \frac{1}{2} \quad \text{against} \quad H_A: \frac{\lambda_1}{\lambda_1 + \lambda_2} > \frac{1}{2}$$

We will test this null hypothesis by using the binomial distribution under this null hypothesis, when the binomial parameter equals $\frac{1}{2}$. If the alternative hypothesis is correct, i.e. the basic frequency for Scenting is the larger one, then Scentings are more frequent than Flehmens and thus t_1 is large compared with s . The test is carried out by adding the binomial probabilities from the observed value t_1 up to the total number of recorded reactions s :

$$UP = \sum_{v=t_1}^s \binom{s}{v} \left(\frac{1}{2}\right)^v \left(1 - \frac{1}{2}\right)^{s-v}$$

UP means Upper Probability. If this probability is smaller than a critical limit, the significance level, to be discussed later in this appendix, we reject the null hypothesis and conclude that the alternative hypothesis is correct, i.e. that the basic frequency for Scenting is higher than that for Flehmen.

As the basic frequency may vary quite considerably depending on category of lion, type of scent mark, location, and experimental conditions, it is necessary to make the comparison of the basic frequencies of Scenting and Flehmen for each combination of lion category, scent mark, location, and experimental condition.

Example 3

Consider the observations for Cubs presented for the scent mark PI Male in the outdoor experiments in Copenhagen. The received presentations amount to $M = 16$. There have been recorded $t_1 = 18$ Scentings and $t_2 = 1$ Flehmen, thus the total number of recorded reactions is $s = 19$. Then we get the Upper Probability 0.00004, which is quite small, and we will conclude that the basic frequency for Scenting is higher than that for Flehmen for this particular experimental set up.

The same test can be carried out for all combinations of the categories of lions and the types of scent marks for all four experiments. The calculations can be carried out by the SAS programme package, by which it is possible to calculate the exact probabilities. In the table *Comparison of Scenting and Flehmen frequencies* the probabilities are reported for all combinations where the probabilities are less than 0.10. In most cases the probabilities are very small and we can conclude that the Scenting frequencies are larger than the Flehmen frequencies.

Tests for comparisons of different categories for the same type of reaction

The basic frequency λ may be different for males, females, and cubs presented to the same scent mark under otherwise the same conditions. Furthermore, λ may also be different when the same category of lions is presented for different scent marks. There may also be differences when the same category of lions is presented to the same scent mark outdoors and indoors.

We will make comparisons of the results of presentations of only two scent marks or only two categories of lions at a time. For such comparisons calculations of exact probabilities can be carried out, if more than two categories were compared at a time,

exact calculations are difficult to perform, and most people will rely on large sample methods, like χ^2 -tests for which the actual numbers of observations are too small.

For these comparisons the number of received presentations will be different for the two experimental set ups we are comparing. To illustrate the notation consider Example 1 above, where the number of Scentings were recorded after presentations of the scent mark PI Male to Males, Females, and Cubs. We start by comparing the basic frequencies for Males and Females. Let the basic frequency for Males be λ_1 and for Females λ_2 . Here there is no apriori indication about which of these basic frequencies that might be the larger one. The null hypothesis to be tested here is $H_0: \lambda_1 = \lambda_2$ against the alternative hypothesis $H_A: \lambda_1 \neq \lambda_2$. This is a *two-sided* alternative hypothesis in contrast to the *one-sided* used in the previous section.

For Males the number of received presentations is $M_1 = 5$ and the recorded number of Scentings is $t_1 = 15$, for Females the received number of presentations is $M_2 = 8$ and the recorded number of Scentings is $t_2 = 8$. The total number of recorded Scentings is $s = t_1 + t_2 (= 23)$, which is Poisson distributed with Poisson parameter $(M_1 \lambda_1 + M_2 \lambda_2)$. The conditional distribution of the number of t_1 , the recorded number of Scentings for Males, given s , the total number of recorded Scentings for the two categories of lions, is binomially distributed with parameters s and

$$\frac{M_1 \lambda_1}{M_1 \lambda_1 + M_2 \lambda_2}$$

which is the binomial parameter. The probability for the result is

$$\binom{s}{t_1} \left(\frac{M_1 \lambda_1}{M_1 \lambda_1 + M_2 \lambda_2} \right)^{t_1} \left(1 - \frac{M_1 \lambda_1}{M_1 \lambda_1 + M_2 \lambda_2} \right)^{s-t_1} \quad \text{where } t_1 = 0, 1, \dots, s$$

If the null hypothesis holds, and the two basic frequencies are equal, then the binomial parameter above will be reduced to $M_1/(M_1 + M_2)$. Thus with the conditional distribution given above the original H_0 and H_A are transformed into the following null hypothesis and alternative hypothesis, respectively:

$$H_0: \frac{M_1 \lambda_1}{M_1 \lambda_1 + M_2 \lambda_2} = \frac{M_1}{M_1 + M_2} \quad \text{against} \quad H_A: \frac{M_1 \lambda_1}{M_1 \lambda_1 + M_2 \lambda_2} \neq \frac{M_1}{M_1 + M_2}$$

If the basic frequency for Males is higher than that for Females, then t_1 will be large compared with s , while if the basic frequency for Males is smaller than that for Females, then t_1 is small compared with s . Thus we reject the null hypothesis H_0 either if t_1 is large or if t_1 is small. The test is performed in the following way: In case t_1 is large we add the binomial probabilities from the observed value t_1 up to the total number of recorded reactions s . This is *UP*, the Upper Probability, and we reject the null hypothesis if this probability is small. In case t_1 is small we add the binomial

probabilities from zero up to and including the observed value t_1 . This is LP , the Lower Probability. If this is small, we reject the null hypothesis. The two probabilities UP and LP are calculated by means of binomial distribution under the null hypothesis. Thus the UP is calculated as follows:

$$UP = \sum_{v=t_1}^s \binom{s}{v} \left(\frac{M_1}{M_1 + M_2} \right)^v \left(1 - \frac{M_1}{M_1 + M_2} \right)^{s-v}$$

The LP is calculated as follows:

$$LP = \sum_{v=0}^{t_1} \binom{s}{v} \left(\frac{M_1}{M_1 + M_2} \right)^v \left(1 - \frac{M_1}{M_1 + M_2} \right)^{s-v}$$

These probabilities can be calculated for all pairwise comparisons. In practise both UP and LP should be calculated for each comparison, and the smaller one of these must be chosen. As the alternative hypothesis is two-sided, i.e. that the basic frequency of males may be smaller or higher than that of the females, the chosen probability is multiplied with two. This value is called the *significance probability*. If this value is less than a certain limit, the null hypothesis has been rejected for the actual comparison, and it is concluded that the basic frequencies are different.

The probabilities can be calculated by means of the SAS programme package by using the binomial probability function. This means that UP has to be calculated in the following way:

$$UP = 1 - \sum_{v=0}^{t_1-1} \binom{s}{v} \left(\frac{M_1}{M_1 + M_2} \right)^v \left(1 - \frac{M_1}{M_1 + M_2} \right)^{s-v}$$

Example 4

We will again refer to the data in Example 1. In the following table we consider the comparisons of Scenting frequencies of Males and Females, Males and Cubs, and Females and Cubs after presentations of the scent mark PI Male at the outdoor experiments in Copenhagen. In the table we give the following figures for each comparison: $M_1/(M_1 + M_2)$, UP , LP , and the significance probability.

Tests for Copenhagen outdoor experiments with PI Male scent mark presentations

Comparison	$M_1/(M_1 + M_2)$	UP	LP	Sign. prob.
Males-Females	0.385	0.00849	0.99752	0.017
Males-Cubs	0.238	0.00522	0.99826	0.010
Females-Cubs	0.333	0.67931	0.48181	0.964

It is seen that the *UP* is the smaller one in two of the three cases. From these results we may conclude that the basic frequency of Males is significantly larger than that of Females and that of Cubs, but that there is no detectable difference between the basic frequencies of Females and Cubs.

Such tests can be carried out for comparisons between the three categories of animals for each type of scent mark in all four experimental series, and for comparisons between the different types of scent marks for each category of lions in all four experimental series. These calculations are done for Scenting and Flehmen, separately.

Significance levels for multiple tests

The statistical analyses consist in multiple pairwise comparisons as illustrated in the example above. For such multiple tests there are problems with the choice of the significance levels of the tests. If we are testing a null hypothesis H_0 against an alternative hypothesis H_A at a significance level 5%, this means that we reject the null hypothesis with a probability of wrongly rejecting this hypothesis, i.e. erroneously to conclude that H_0 does not hold and that the alternative hypothesis H_A is correct. We have chosen the level of significance and the critical limits such that even though the null hypothesis is correct, then 5% of the observations will be so extreme that they will fall beyond the critical limits and lead to the rejection of the hypothesis. On the other hand 95% of the observations will fall within the critical limits and will not lead to any rejection of the null hypothesis.

Let us assume that we want to carry out 5 independent tests of null hypotheses, each one at the level 5%. Assume that all 5 hypotheses are correct. Then the probability that none of these hypotheses are rejected will be $(0.95)^5$. The probability for wrongly rejecting at least one of these 5 null hypotheses will then be $1 - (0.95)^5 = 1 - 0.7738 = 0.2262$. This means that the total level of significance for this set of null hypotheses will be 22.62% and not 5%. This example illustrates that when we are carrying out multiple tests, the probability of erroneously rejecting at least one of the hypotheses, will increase strongly with the number of tests. If we instead choose 1% as the significance level of the individual tests the total significance level will be $1 - (0.99)^5 = 1 - 0.9510 = 0.0490$. Thus by reducing the level of significance for the individual tests the total significance level will also be decreased.

Our pairwise comparisons, as in the example above, are not independent. It can be shown that if we have k individual tests each with a significance level α , then the probability for wrongly rejecting at least one of them will be less than $k\alpha$. Thus if we decide that the total significance level for a set of tests should be α_0 , and we want all individual tests to have the same significance level, then the significance level for the individual tests will be α_0/k .

We reject the null hypothesis of an individual test if the *significance probability* calculated as described in the previous section *is less than* the chosen *significance level*

Example 5

In Example 4 we made 3 pairwise comparisons. If we here choose the total significance level 5%, the significance levels of the individual tests will be $5/3 = 1.667\%$. It is seen that the significance probability for the comparison Males-Cubs is below limit, and we reject the null hypothesis in this case. The comparison Males-Females is a border line case, as the significance probability is very near to the significance level, while for the comparison Females-Cubs we accept the null hypothesis.

For comparisons of the three categories of animals, the significance levels of the individual tests will be one third of the total significance level. For comparisons of effects of 5 different scent marks, we carry out in all 10 pairwise comparisons. Thus for these tests the significance level of the individual tests will be one tenth of the total significance level. Finally, for the comparison of 4 scent marks, as in Givskud outdoors, there are six pairwise comparisons and the significance level for the individual tests is a sixth of the total level of significance.

Pooling of individuals into groups

The groups of individuals considered so far are all those belonging to the same category of animals and have been presented for the same scent mark in one of the four experimental series. In the two outdoor series there are different females, which have been observed individually. In the analysis considered here they have been pooled into one group after statistical tests of the types considered in this appendix had showed that their basic frequencies were not significantly different.

Appendix 3. Scent presentation data by location for lions, tigers and leopards.

A: Lion data:

Location of scent presentations: Copenhagen Outdoor

Observations

Number of received stimuli:

	Lion male	Lion female	Lion female estrous	Tiger	Leopard
Male	5	3	5	10	5
Female	8	4	7	20	10
Cub	16	8	16	40	20

Number of Scenting observed

	Lion male	Lion female	Lion female estrous	Tiger	Leopard
Male	15	7	9	25	2
Female	8	5	7	31	2
Cub	18	6	7	54	13

Number of Flehmen observed

	Lion male	Lion female	Lion female estrous	Tiger	Leopard
Male	0	0	1	0	0
Female	2	0	4	9	2
Cub	1	1	3	11	8

Estimated Frequency Parameters:

Scenting

	Lion male	Lion female	Lion female estrous	Tiger	Leopard
Male	3.000	2.333	1.800	2.500	0.400
Female	1.000	1.250	1.000	1.550	0.200
Cub	1.125	0.750	0.438	1.350	0.650

Flehmen

	Lion male	Lion female	Lion female estrous	Tiger	Leopard
Male	0	0	0.200	0	0
Female	0.250	0	0.571	0.450	0.200
Cub	0.063	0.125	0.188	0.275	0.400

Location of scent presentations: Copenhagen Indoor

Observations

Number of received stimuli:

	Lion male	Lion female	Lion female estrous	Tiger	Leopard
Male	15	18	10	3	3
Female	30	36	20	6	6
Cub	60	72	40	12	12

Number of Scenting observed

	Lion male	Lion female	Lion female estrous	Tiger	Leopard
Male	12	9	11	4	5
Female	28	26	15	4	2
Cub	27	45	22	22	8

Number of Flehmen observed

	Lion male	Lion female	Lion female estrous	Tiger	Leopard
Male	0	1	0	0	1
Female	0	2	2	1	0
Cub	3	8	5	8	2

Estimated Frequency Parameters:

Scenting

	Lion male	Lion female	Lion female estrous	Tiger	Leopard
Male	0.800	0.500	1.100	1.333	1.667
Female	0.933	0.722	0.750	0.667	0.333
Cub	0.450	0.625	0.550	1.833	0.667

Flehmen

	Lion male	Lion female	Lion female estrous	Tiger	Leopard
Male	0.000	0.056	0.000	0.000	0.333
Female	0.000	0.056	0.100	0.167	0.000
Cub	0.050	0.111	0.125	0.667	0.167

Location of scent presentations: Givskud Outdoor

Observations

Number of received stimuli:

	Lion male	Lion female	Lion female estrous	Tiger	Leopard
Male	15	8	-	4	2
Female	43	26	-	10	5
Cub	84	48	-	36	12

Number of Scenting observed

	Lion male	Lion female	Lion female estrous	Tiger	Leopard
Male	4	0	-	3	0
Female	8	2	-	10	4
Cub	11	5	-	8	3

Number of Flehmen observed

	Lion male	Lion female	Lion female estrous	Tiger	Leopard
Male	2	0	-	3	0
Female	0	2	-	3	3
Cub	2	1	-	2	1

Estimated Frequency Parameters:

Scenting

	Lion male	Lion female	Lion female estrous	Tiger	Leopard
Male	0.267	0.000	-	0.750	0.000
Female	0.186	0.077	-	1.000	0.800
Cub	0.131	0.104	-	0.222	0.250

Flehmen

	Lion male	Lion female	Lion female estrous	Tiger	Leopard
Male	0.133	0.000	-	0.750	0.000
Female	0.000	0.077	-	0.300	0.600
Cub	0.024	0.021	-	0.056	0.083

Location of scent presentations: Givskud Indoor

Observations

Number of received stimuli:

	Lion male	Lion female	Lion female estrous	Tiger	Leopard
Male	30	36	6	20	12
Female	136	160	40	80	48
Cub	144	132	12	120	72

Number of Scenting observed

	Lion male	Lion female	Lion female estrous	Tiger	Leopard
Male	3	6	2	6	3
Female	9	10	9	15	8
Cub	4	5	0	21	9

Number of Flehmen observed

	Lion male	Lion female	Lion female estrous	Tiger	Leopard
Male	0	0	1	3	2
Female	0	0	0	6	4
Cub	0	0	0	12	4

Estimated Frequency Parameters:

Scenting

	Lion male	Lion female	Lion female estrous	Tiger	Leopard
Male	0.100	0.167	0.333	0.300	0.250
Female	0.066	0.063	0.225	0.188	0.167
Cub	0.028	0.038	0.000	0.175	0.125

Flehmen

	Lion male	Lion female	Lion female estrous	Tiger	Leopard
Male	0.000	0.000	0.167	0.150	0.167
Female	0.000	0.000	0.000	0.075	0.083
Cub	0.000	0.000	0.000	0.100	0.056

B: Tiger data.

Location of scent presentations: Copenhagen Outdoor

Observations

Number of received stimuli:

	Tiger male	Tiger female	Tiger female estrous	Lion	Leopard
Male	3	3	4	3	-
Female	3	4	2	2	-

Number of Scenting observed

	Tiger male	Tiger female	Tiger female estrous	Lion	Leopard
Male	57	19	29	20	-
Female	9	14	4	6	-

Number of Flehmen observed

	Tiger male	Tiger female	Tiger female estrous	Lion	Leopard
Male	13	6	10	6	-
Female	0	0	0	0	-

Estimated Frequency Parameters:

Scenting

	Tiger male	Tiger female	Tiger female estrous	Lion	Leopard
Male	19.00	6.33	7.25	6.67	-
Female	3.00	3.50	2.00	3.00	-

Flehmen

	Tiger male	Tiger female	Tiger female estrous	Lion	Leopard
Male	4.33	2.00	2.50	2.00	-
Female	0.00	0.00	0.00	0.00	-

Location of scent presentations: Copenhagen Indoor

Observations

Number of received stimuli:

	Tiger male	Tiger female	Tiger female estrous	Lion	Leopard
Male	17	18	4	12	3

Number of Scenting observed

	Tiger male	Tiger female	Tiger female estrous	Lion	Leopard
Male	109	85	24	74	21

Number of Flehmen observed

	Tiger male	Tiger female	Tiger female estrous	Lion	Leopard
Male	14	5	2	2	1

Estimated Frequency Parameters:

Scenting

	Tiger male	Tiger female	Tiger female estrous	Lion	Leopard
Male	6.41	4.72	6.00	6.17	7.00

Flehmen

	Tiger male	Tiger female	Tiger female estrous	Lion	Leopard
Male	0.82	0.28	0.50	0.17	0.33

Location of scent presentations: Knuthenborg Outdoor

Observations

Number of received stimuli:

	Tiger male	Tiger female	Tiger female estrous	Lion	Leopard
Castrate	18	-	-	21	-
Female	6	-	-	3	-
Cub	24	-	-	28	-

Number of Scenting observed

	Tiger male	Tiger female	Tiger female estrous	Lion	Leopard
Castrate	0	-	-	51	-
Female	14	-	-	14	-
Cub	7	-	-	17	-

Number of Flehmen observed

	Tiger male	Tiger female	Tiger female estrous	Lion	Leopard
Castrate	0	-	-	16	-
Female	3	-	-	4	-
Cub	7	-	-	7	-

Estimated Frequency Parameters:

Scenting

	Tiger male	Tiger female	Tiger female estrous	Lion	Leopard
Castrate	0.00	-	-	2.43	-
Female	2.33	-	-	4.67	-
Cub	0.29	-	-	0.61	-

Flehmen

	Tiger male	Tiger female	Tiger female estrous	Lion	Leopard
Castrate	0.00	-	-	0.76	-
Female	0.50	-	-	1.33	-
Cub	0.29	-	-	0.25	-

C: Leopard data.

Location of scent presentations: Copenhagen Outdoor

Observations

Number of received stimuli:

	Leopard male	Leopard female	Lion	Tiger
Male	3	-	5	5
Female	3	-	5	5
Cub	6	-	10	10

Number of Scenting observed

	Leopard male	Leopard female	Lion	Tiger
Male	4	-	13	13
Female	4	-	5	9
Cub	2	-	4	2

Number of Flehmen observed

	Leopard male	Leopard female	Lion	Tiger
Male	2	-	7	5
Female	3	-	3	3
Cub	1	-	1	0

Estimated Frequency Parameters:

Scenting

	Leopard male	Leopard female	Lion	Tiger
Male	1.33	-	2.60	2.60
Female	1.33	-	1.00	1.80
Cub	0.33	-	0.40	0.20

Flehmen

	Leopard male	Leopard female	Lion	Tiger
Male	0.67	-	1.40	1.00
Female	1.00	-	0.60	0.60
Cub	0.17	-	0.10	0.00

Location of scent presentations: Copenhagen Indoor

Observations

Number of received stimuli:

	Leopard male	Leopard female	Lion	Tiger
Male	6	3	7	5
Female	6	3	7	5
Cub	12	6	14	10

Number of Scenting observed

	Leopard male	Leopard female	Lion	Tiger
Male	5	0	5	3
Female	10	2	6	1
Cub	2	2	1	0

Number of Flehmen observed

	Leopard male	Leopard female	Lion	Tiger
Male	2	0	2	1
Female	4	0	2	0
Cub	0	0	0	0

Estimated Frequency Parameters:

Scenting

	Leopard male	Leopard female	Lion	Tiger
Male	0.83	0.00	0.71	0.60
Female	1.67	0.67	0.86	0.20
Cub	0.17	0.33	0.07	0.00

Flehmen

	Leopard male	Leopard female	Lion	Tiger
Male	0.33	0.00	0.29	0.20
Female	0.67	0.00	0.29	0.00
Cub	0.00	0.00	0.00	0.00

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