THE INFLUENCE OF FAECAL SCENT MARKS ON THE BEHAVIOUR OF THE WHITE RHINOCEROS (*CERATOTHERIUM SIMUM SIMUM*)

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Abstract

From September 2005 to March 2006 a zoo study was performed with one male and two female rhinos at Orana Wildlife Park in Christchurch. The study had two aims: (1) to assess whether faeces from unfamiliar rhinos carry information that influences the behaviour of adult rhinos in a zoo habitat, and (2) to identify olfactory constituents of the faeces that potentially stimulate the change in behaviour.

Faeces samples were collected from seven male, female, and juvenile rhinos residing at Hamilton and Auckland zoos and from one male rhino held in a separate enclosure at Orana Park. From each sample type six individual samples of 2 kg each were collected. The samples were put in plastic bags and kept frozen at –10°C. As controls, samples from peat, peat with testosterone, and heated faeces were used.

An individual thawed sample was presented at a time to the subjects by placing it into the enclosure. Behaviour and positions of the subjects was monitored for 3 h. In addition weather data was collected.

Each exposure test of one sample type consisted of at least six observation sessions during which one two-kg dung specimen was presented to the subjects in the Orana Park enclosure. Each session consisted of nine trial periods of 15 min, during which each subject was observed individually and all actions of the animal were recorded.

There was a strong response of the subjects to faeces from male donors. The bull reacted with increased frequency of spray urinating. The bull habituated to the faecal stimulus within about an hour. Other strongly affected behaviour categories of all subjects included smelling the ground,

scanning, and walking. The distance between the subjects and frequency of synchronous behaviour of the subjects was affected by various samples to a lesser degree.

Correlation of faecal compounds with territorial behaviour activities and chemical analysis identified possible signalling compounds such as esters of low molecular weight fatty acids (propanoic, butanoic, and pentanoic acids) and perhaps testosterone and terpenoids. Even simulated control samples consisting of peat impregnated with testosterone initiated some response.

Overall, faecal scent marks were found to stimulate multifaceted behavioural effects of captive white rhinoceros. Chemical analyses of faeces identified new olfactory components not previously attributed to male faeces, and which could act as male signalling compounds.

Chapter 1

1. Introduction

The southern white rhinoceros (*Ceratotherium simum simum*, called hereafter 'white rhino' or just 'rhino') is one of the last large animals that still exist on Earth. Their longevity and low reproductive rate make them highly vulnerable to human exploitation. The white rhino was on the brink of extinction by the end of the 19th century, due to hunting, poaching, and loss of habitat. Intensive protection and extensive translocations of individual animals into small nature reserves and privately owned land led to a recovery of the population (Emslie & Brooks, 1999; Pienaar, 1970; Rookmaaker, 2000; WWF, 2006). The total population was estimated at 12,080 in 2005, with 740 of those being individuals living in captivity (International-Rhino-Foundation, 2006; Owen-Smith, 1973). Today, 90 % of all white rhinos in Africa live in South Africa (Emslie & Brooks, 1999). This concentration of the population in one country is risky as political instability could jeopardise present anti-poaching programmes and lead again to the decimation of the population.

The white rhino is the third largest land animal after the Asian and African elephants, standing up to 1.8 m tall at the shoulder and weighing up to 2,700 kg (International-Rhino-Foundation, 2006). Rhinos belong to the order Perissodactylae or odd-toed ungulates, a group which also includes horses Equidae and tapir Tapiridae. The common name of white rhino originated in South Africa from the Afrikaans word "wijd" describing its wide mouth and not its colour. Their wide, square lips, as well as size, distinguish them from the smaller, prehensile lipped (adapted for feeding from trees and shrubs) black rhinoceros. White rhinos are divided into the northern and southern subspecies, which appear similar but have been genetically separated for two million years (Emslie & Brooks, 1999; Estes, 1991; Grzimek, 1990; Hillman-Smith et al., 1986).

The white rhinoceros is found in the long- and short-grass savannahs and woodlands of southern and central Africa. It requires relatively flat terrain, bush for cover, grass for grazing and water for drinking and wallowing in. White rhinos have no incisors or canine teeth and use their square lips to graze or browse large areas of grassland.

Adult female white rhinoceroses bear their first calf when they are six or seven years old, after a gestation period of approximately 16 months. A single calf is born about every two to four years. Males reach sexual maturity between ten and twelve years of age. The calves are weaned at about one year of age.

White rhinos, although more sociable than other species of rhino, are primarily solitary, requiring large individual areas because of their size and daily food requirements. Males are quite territorial and are considered solitary beings while females tend to associate in groups. In the wild, an average home range is approximately 80-260 ha and can contain six or seven territories. This means that there is only enough space for two out of three adult males to have their own territory within the social group's home range (ThinkQuest, 2002).

The study of the reproductive behaviour of the white rhinos is important for the conservation of free-living populations because, today, free-living white rhinos exist only in small discrete populations. On average only 40 individuals live in a single wild population (Emslie & Brooks, 1999) and, therefore, inbreeding is potentially a serious problem. Greater knowledge could help in assessing the number of breeding animals in these populations, and in assessing the level of inbreeding (Parker & Waite, 1997; Schreiber et al., 1995). There is intense intrasexual competition for mates and strict criteria for mate choice in white rhinos, as only a small number of all possible breeding animals in a population actually reproduce. Populations with only a few breeding individuals need to be carefully managed, to conserve genetic diversity, Identifying reproductively successful males and exchanging them between populations is potentially an important step toward successful management (Andersson, 1994; Møller & Jennions, 2001; Trivers, 1972).

Dominant adult males maintain strict, non-overlapping ranges ('territories') and defend these territories to ensure adequate food and minimal reproductive competition. The feeding ranges of females overlap six to seven male territories, and females may wander freely through these territories without threatening the dominant males. Other males may graze within the territory of a dominant male provided they display submissive behaviours when challenged. Alpha bulls are usually older and have higher androgen levels than beta bulls (Rachlow et al., 1998). They successfully defend a territory while beta bulls adopt a subordinate status (Owen-Smith, 1973; Owen-Smith, 1975; Rachlow et al., 1998). The territorial bull marks his territory by spray urinating, scattering his dung at muckheaps and dragging his feet along the ground. This behaviour is not tolerated from other non-dominant bulls. Satellite males may share bathing pools and wallows, as these resources are scarce and in high demand; however, these non dominant males feed and sleep separately from the group. Confrontations between dominant males occur at territorial boundaries, but these seldom result in injury. Males may also fight over of a female in oestrus, and these fights are more likely to end in injury or death.

Given that females seem to have their own territories and that the territories of males overlap with multiple females, it is likely that males compete for the best territories because these will be more likely to coincide with the highest quality females. Therefore, territory quality may not be an issue for females when choosing a mate. Indeed females may have little choice because access to alternative males is restricted by the alpha male. Only in those cases where a female's territory overlaps with one or more male territories would she have a choice.

Scent marks are a very common form of signalling by male mammals and they tend to function as sign posts (i.e. they can potentially give information to receivers in the absence of the signaller). The simplest information conveyed is that some other individual has been present in the area. However, it is known that a variety of mammals can use scent marks for identifying the particular individual that made the mark (Johnson, 1973). Generally, scent marks of dominant males have stronger aversive properties than those of subordinate males, but intrinsic information may not be sufficient for deciding whether or not to leave a scent-marked area.

The scent-matching hypothesis of Gosling and McKay (1990) may be applicable to the white rhino. One prediction from this hypothesis is that when males compare their odour with that of scent marks in the vicinity they will be more reluctant to fight if these odours are the same.

The competing countermark hypothesis (Rich & Hurst, 1998) may also be applicable to white rhinos. "Countermarking" or "overmarking" is a common behaviour in mammals (Gosling, 1982). One animal places its own marks next to or on the competitor's scent mark. The hypothesis is that countermarking facilitates detection of asymmetry between the two males and thereby a male can show his status and avoid actual fights with intruders. Another possibility is that females use marking sites to assess potential mates. By comparing the scent of samples with that of other males, the females may recognise the state (age, dominance, health) of the male and make optimal mate-choice decisions.

Captive populations could act as a safety net for conservation, but white rhinos do not reproduce very well in captivity (Gunn et al., 1998; Rieches, 1998; Schwarzenberger et al., 1999). The reasons for low reproductive rates are still not fully understood, but a likely hypothesis is that it is related to unnatural social relationships in the captive setting. Lindemann (1982) found that the main requirement for successful breeding is the presence of more than one male. Male-male competition seems necessary for the stimulation of male sexual behaviour while also potentially initiating oestrous cycling in females present in the group (Owen-Smith, 1988). Because of the absence of male-male competition in zoos, territorial behaviour is largely suppressed. Other factors also influence the breeding behaviour of white rhinos, as even large mixed groups kept in captivity often fail to reproduce (Gunn et al., 1998; Rieches, 1998). Nutritional deficiencies or a lack of seasonal variation in food supply have been shown to influence hormone balance in male and female white rhinos (Gunn et al., 1998) and hormone imbalance may in turn cause infertility. As an indication of how serious the problem may be, in 1996 the reproduction rate in captivity of the F1- and F2-generations was only 8 % and 0 %, respectively (Schwarzenberger et al., 1999). Absence of mate choice options could lead to genetic incompatibility and thus reduce fertilization success.

The problem of white rhinos not breeding well in captivity has led to expensive measures, especially shipping white rhinos between different enclosures (Ruempler, 1991). Having identified rhinos as territorial and highly olfactory mammals that normally living in groups where intrasexual competition is critical, it might be that the introduction of foreign male faeces could influence the sexual dynamics of a captive breeding group. If it could be shown that excrement carries sufficient information to influence rhino behaviour by simulating the presence of other animals, this could offer a more financially viable means of dealing with the problems of

breeding of white rhinos in captivity. At the same time it could lead to improving environmental enrichment methods by providing stimuli to promote rhino-specific behavioural activities in an understimulating environment (Reinhardt, 1999).Specific questions in this study are listed below.

1. Do faeces from foreign males influence the marking behaviour of territorial white rhino males?

    a) Is the vigilance of territorial bulls increased when they find foreign faeces in their territory?

    b) Do territorial bulls mark more often when they find foreign faeces in their territory?

    c) Do territorial bulls mark in the vicinity of foreign faeces or even cover faeces?

    d) Can males distinguish between faeces of other males, females, juveniles, and peat (a control to see whether it is specifically faeces that influences the male) and how do they respond (e.g. do they show a change in scent marking behaviour)?

    e) Does the male habituate to the foreign faeces?

2. Is the activity of white rhinos affected by the introduction of foreign faeces?

    a) Do they show enhanced motion?

    b) Do they flock together?

    c) Do they synchronize their behaviour?

3. Do white rhinos recognise different components in different faeces?

    a) Is the response of females and males a function of hormone or pheromone levels in the foreign faeces?

    b) Can compounds that affect rhino behaviour be chemically identified?

4. Is the response of females and males a function of weather conditions?

This study may be important in terms of captive breeding husbandry and behavioural enrichments. Observations of the interactions that result may provide possible solutions to breeding problems and take steps towards securing future populations of white rhinos.

Chapter 2

2. Materials and Methods

Experiments were performed with three resident white rhinos (one adult male and two adult females) at Orana Park. These three individuals will be referred to as 'subjects'. The basic testing method was to introduce dung samples into the habitat of the subjects, after which the subjects' behaviour was recorded.

Faeces originated from male, female, and juvenile white rhinos at three zoos: Orana Wildlife Park, Christchurch, Hamilton Zoo, and Auckland Zoological Park. These individuals will be referred to as 'donors'.

2.1. Observed Animals

All three subjects (Figs. 2.1. and 2.2.) were born in captivity. A fourth adult bull living separated from the three at Orana Park was used only as donor (see below). This animal stayed in an extra enclosure which was not connected to the main enclosure and was not accessible to the public. Table 2.1. gives lineage information on the subjects mostly obtained from the rhino studbook. The rhino studbook is a record of the history of the captive rhino population. It includes pedigrees of animals and a listing of the various locations in which animals have been held. The studbook traces the entire history of each individual in a population with each individual having its own studbook number. The code given in Table 2.1 will be used in the following as an abbreviation for the subjects.

Figure 2.1. Cyrano, male white rhino at Orana Park

Figure 2.2 Utani (foreground) and Mapensi, female white rhinos at Orana Park.

Table 2.1. Code and lineage details for white rhinos observed at Orana Park

Code	NAME	SEX	STUD-BOOK	BIRTHDATE	BIRTH LOCATION	SIRE	DAM
CY	Cyrano	♂	921	23.01.1987 – 19 years	JAX	390 - JAX	391 - JAX
UT	Utani	♀	820	08.07.1984 – 21 years	SDWAP	Mandhla - SDWAP	Umfolozi - SDWAP
MP	Mapenzi	♀	821	29.07.1984 – 21years	SDWAP	Mandhla - SDWAP	Macite - SDWAP

JAX = Jacksonville Zoological Gardens – USA
SDWAP = San Diego Wild Animal Park – USA

2.2. The Rhino Enclosure

The subjects normally spent the day at Orana Park in a large outdoor enclosure (approximately 200 m x 100 m) (Fig. 2.3.) where the terrain sloped slightly from southwest to northeast. A steel rope and an electric fence surrounded the enclosure. There was a visitor's path between the northeast side of the enclosure and a pond (width of pond, 8 m). Trees on three sides of the enclosure and inside the enclosure provided the subjects with access to shade and shelter from rain. There were logs in the enclosure, serving to protect a tree island on the right and there was a steel-rope fence that protected a bush island on the left.

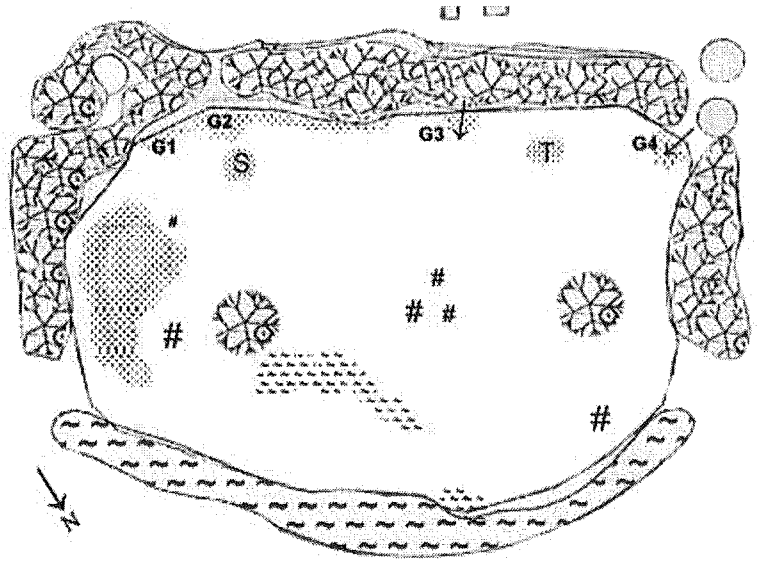

Figure 2.3. Plan of the rhino enclosure at Orana Park. The entrance gates are numbered G1 through G4, sample position S, and toilet T (for further explanations, see text).

The enclosure has four gates. The entrance to the night enclosures of the females (G4) was situated in the western corner. The right-hand gate (G3) on the south-western side led to the male night enclosure. The left gate on the south-western side was the keepers' entrance (G2). In the southern corner lay the gate which led to the night enclosure of the second male and the giraffe enclosure (G1). This gate is only used for maintenance purposes and in the rare case the male rhinos get swapped. At the northern corner was the public feeding area for visitors, which was open once a day at 3 PM.

The terrain was primarily grass with some sand patches close to the gates and a big sand area in the southeast. Depending on the weather there was a wet mud pit on the northwest side. Several logs, big roots and dead trees were used as enrichment.

The subjects each had a night enclosure of approximately 500m². Each enclosure had a water- and windproof shelter with sawdust and straw. The ground itself was sand. A watering place, a toilet hole and behaviour enrichment equipment e.g. a log, and truck tire (hung up or on the floor) completed the night enclosures.

The rhinos were kept with six scimitar-horned oryx (*Oryx dammah,* a highly endangered antelope from central Niger and Chad) in the same enclosure (Orana-Wildlife-Trust, 2006).

2.3. Sample collection

The tests included samples from foreign donors (Table 2.2) and various control samples. Overall I conducted 13 tests (Fig. 2.4), each with different dung sample types (for a detailed description, see next section Table 2.3):

- four sample types from different foreign donor bulls
- three sample types from different foreign donor cows
- two sample types from different donor juveniles
- one heated 'control' sample type
- one peat sample type

– one peat sample type with testosterone added

– one 'control sample' type (i.e. no sample was used)

Each sample type consisted of at least six individual dung specimens of two kg each.

Table 2.2. Information on donor animals from which faeces samples were collected.

NAME	SEX	STUD-BOOK	DATE OF BIRTH	HOLDER	BIRTH LOCATION	SIRE	DAM
Stumpy	♂	801	17.07.1983 - 22Y	OWP	DUBBO	Homas - DUBBO	Nandi - DUBBO
Zambese	♂	1356	~ 1992 - 21Y	HZNZ	KNP	Wild - NPSA	Wild - NPSA
Moesha	♀	1357	~ 1994 - 12Y	HZNZ	KNP	Wild - NPSA	Wild - NPSA
Caballe	♀	1358	~ 1995 - 11Y	HZNZ	KNP	Wild - NPSA	Wild - NPSA
Inkosi	♂	1409	06.01.2002 - 4Y	HZNZ	HZNZ	Zambese - HZNZ	Caballe - HZNZ
Mtoto	♂	1502	17.02.2004 - 2Y	HZNZ	HZNZ	Zambese - HZNZ	Caballe - HZNZ
Kito	♀	1353	26.06.2000 - 5Y	HZNZ	AZP	Wild - KNP	Mazithi - AZP
Mandhla	♂	541	05.05.1979 - 27Y	AZP	SDWAP	Mandhla - SDWAP	Umfolozi - SDWAP
Kruger	♂	1273	~ 1989 - 16Y	AZP	KNP	Wild - KNP	Wold - KNP

OWP = Orana Wildlife Park Christchurch – New Zealand
DUBBO = Western Plains Zoo NSW – Australia
HZNZ = Hamilton Zoo – New Zealand
KNP = Kruger National Park – South Africa
NPSA = Natal Park – South Africa
AZP = Auckland Zoological Park – New Zealand
SDWAP = San Diego Wild Animal Park – USA

Dung samples from each donor were collected over a 7-week period (2 kg per week, 0.5 kg for analysis per week). Such a long collection period was chosen in order to average-out variations in the hormone levels of donors due to oestrus cycle (Schwarzenberger et al., 1998) and other short-term influences (Udo Ganslosser, private communication). The last sample (seventh week) served as a backup sample (reserve) just in case one test had to be repeated. Twice 2 kg were collected from each donor bull, one duplicate being a reserve. The duplicates of one donor were used as

Table 2.3. Information on samples. Sample number, donor, sample name, collection period and test period

#	ANIMAL NAME	SAMPLES	COLLECTION PERIOD	EXPERIMENT PERIOD
1.	Stumpy	CHC	06.04. - 18.05.2005	14.11. - 19.11. 2005
2.	Zambese	HAM A	26.04. - 07.06.2005	10.02. - 15.02. 2006
3.	Moesha	HAM X	26.04. - 07.06.2005	31.10. - 05.11. 2005
4.	Caballe	HAM Y	26.04. - 07.06.2005	07.10. - 14.10. 2005
5.	Kito	HAM Z	26.04. - 07.06.2005	22.10. - 29.10. 2005
6.	Inkosi	HAM b	26.04. - 07.06.2005	07.11. - 12.11. 2005
7.	Mtoto	HAM c	26.04. - 07.06.2005	16.10. - 21.10. 2005
8.	Mandhla	AUC A	14.05. - 26.05.2005	21.11. - 01.12. 2005
9.	Kruger	AUC B	14.05. - 26.05.2005	01.02. - 07.02. 2006
10.	Mandhla	AUC-H (heated)	14.05. - 26.05.2005	06.03. - 11.03. 2006
11.	n.a.	Control	n.a.	03.09. - 06.10. 2005
12.	n.a.	Peat	n.a.	02.12. - 04.12. 2005 and 25.01. - 27.01. 2006
13.	n.a.	TestoPeat	n.a.	18.03. - 22.03. 2006

n.a.: not applicable

heated "control" samples. This totalled 91 individual dung samples plus 13 analysis samples (approximately 200 kg of faeces).

In each zoo fresh faeces were collected from each donor in the night shelter once per week on seven consecutive weeks. The faeces were kept in plastic bags and frozen at –10°C. At this temperature the scent is durable for several months (Apps et al., 1989; Roberts, 1998).

A heated control sample was used to test the hypothesis that testosterone or proteins are responsible for the subjects' reaction towards the faeces. The reserve samples of the donor (AUC A) which stimulated a strong reaction in the Orana Wildlife Park population were used. These samples were heated in an oven at 70°C for 24 hours to denature the proteins. The heating was performed in sealed plastic bags resulting in a loss of moisture that was less than 0.7%.

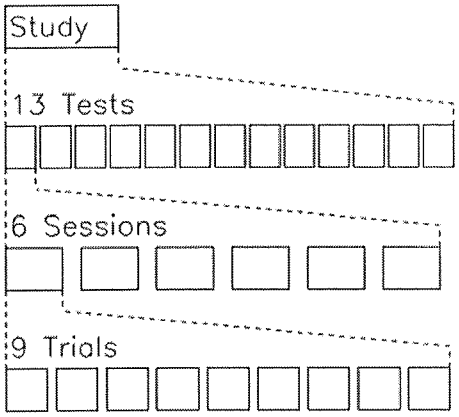

Figure 2.4. Study schematics. The study consisted of tests with 13 different sample types. Each test consisted of six observation sessions (one per day). During one session nine observation trials of individual animals were performed.

For testing whether testosterone alone is responsible for the subjects' reaction, a peat sample impregnated with testosterone was prepared. For the peat samples with testosterone (a concentration of 200 ng/g simulates a 'super bull', (private communication Petra Kretzmar, 2002), 3.1 mg testosterone (4-Androsten-17β-ol-3-one) were dissolved in 1 ml of Methanol and then diluted in 0.5 l of distilled water. With a spray bottle the dissolved testosterone water was spread over and mixed in 15 kg peat.

The untreated peat samples and the peat samples with testosterone were also frozen in 2 kg portions. Because all samples were treated identically it was assumed that there was no systematic difference in the samples. The dung samples were transported by car from Auckland and Hamilton to Christchurch in a frozen state in Styrofoam boxes (cooled to about -20°C with dry ice). At Christchurch the samples were stored in a freezer at -18°C.

2.4. Testing procedure

Dung samples were presented to the subjects in the enclosure by placing a specific specimen of a certain faeces sample type (i.e. from a specific donor) at a predefined position. Each exposure test of one sample type consisted of at least six observation sessions during which one two-kg dung specimen was presented to the subjects in the Orana Park enclosure. One observation session was

carried out per day, beginning in the morning at about 8:30 and ending at about noon. The sample was thawed in an air-tight plastic bucket 24 hours before the start of each session. Each day during one test period a new sample from the same donor was used. The sample was placed in the enclosure before the white subjects were released from the night shelter. The dung sample was presented in the form of a 'cowpat' of about 30 cm in diameter (Fig. 2.5). The position of the samples in the enclosure was the same in all tests. In the evening after the subjects left the enclosure the remainder of the exposed samples together with faeces from the Christchurch subjects were removed.

Figure 2.5. Dung sample (ca. 2 kg) exposed into the Rhino enclosure.

Before testing with a sample, surface layer material at the sample site was replaced by new sand from the surrounding area. The rationale for doing this was to minimise risk of contaminated soil influence how subjects responded to subsequent samples. Testing was cancelled on days when

there was heavy, as rain was likely to wash away sample material and contaminate the surrounding soil.

The above procedure was repeated for each test with a different sample type. Thirteen one week-long tests were performed from September 2005 through March 2006.

2.5. Observation methods

Typically there were six observation sessions for each test (Fig. 2.4), each session lasting 3 h. An observation session started at about 8.30 AM (i.e., after the subjects were released into the enclosure). Data on behaviour were collected over a period of 79 days. Each session consisted of nine trial periods, with there being 15 min of focused observation of a single subject during this period (focal animal method; (Altmann, 1974); see below). With a rolling system a different subject was observed first on each specific day. An example of the sequence of observation trials for the three subjects in the zoo follows: on the first day, CY, UT, MP, CY, UT, MP, CY, UT, MP: on the second day, UT, MP, CY, UT, MP, CY, UT, MP, CY; on the third day, MP, CY, UT, MP, CY, UT, MP, CY, UT and so on. This way I was sure that each subject was observed at each of the trial periods during an observation session. Starting on 14 November 2005, the sequence was changed so that the male rhino, Cyrano, was observed during all trials in parallel with observing the females. The trial sequence for the first day session was: subject CY, CYUT, CYMP, CY, CYUT, CYMP, CY, CYUT, CYMP, and so on, where CYUT means parallel observations of subjects CY and UT during a single 15 min trial period.

Interspersed with the focused observation of individual subjects, a scanning method ((Altmann, 1974), see below) was used to monitor the behaviour and the positions of all subjects at a given time. The time needed for an individual scan was about five minutes. There was also a camera mounted for recording all occurrences of behaviour near the samples (camera focused on sample spot and running as long a subject was nearby.

Besides the behaviour and positions of the subjects, other data were collected: environmental conditions, and the hormone and olfactory composition of the samples.

2.6. Sampling methods for recording behaviour

The sampling methods are as defined by (Altmann, 1974)

2.6.1. Focal animal sampling

Each subject was observed individually for 15 min and all actions by the focal animal were recorded.

2.6.2. Scan sampling method

Between the observations were focused on a single subject, i.e. every 20 min the positions and behaviour of all subjects in the enclosure were recorded. For the purpose of quantifying the positions the enclosure was divided into 8 about equal sized sectors and the sector number in which the subject stayed was recorded.

2.6.3. All occurrence method

In parallel to the above sampling methods, a digital camera recorded every behaviour near the faeces sample regardless of the individual performing the behaviour.

2.6.4. Behaviour catalogue

Following previous studies (Aberham, 2001; Kolar, 2002) I used a modified behaviour catalogue (Handtrack, 1997; Meister, 1997).

I distinguished 33 behavioural categories grouped in six category themes (Table 2.4). There are non-overlapping state behaviour categories like feeding that last for a certain time period and there are instantaneous behaviour categories that are performed simultaneously with a state behaviour, e.g. spray urinating during standing.

Table 2.4. Behaviour categories. Six themes and 33 categories.

CATEGORY THEMES	CATEGORY NAMES	TYPE OF EVENT	DESCRIPTION
Locomotion	Walk	state	Four beat gait with all four feet moved at least once sequentially.
	Trot	state	Two beat gait in which one foreleg and the opposite hind leg are moved at the same time.
	Run	state	Canter or gallop. Three and four beat gaits, respectively, characterized by a moment of suspension.
	Turn	instant	Quick turn, small amount of steps.
Resting/Feeding	Feeding	state	Rhino obtains food, either grazing on grass or on hay and lucernes presented by the keeper
	Drinking	state	Consuming a liquid through the mouth.
	Standing	state	Without motion but with lowered head. No attention to any particular object
	Lying	state	Positioned flat on the side, often with neck and legs outstretched, and with the head usually contacting the ground or lying on the sternum, usually with head up and legs flexed.
Territory protection	Scanning	state	Head elevated (at least 40cm above ground) and ears are pointed.
	Smell sample	state	Sniffing the sample. Distance less than 5 cm.
	Smell ground	state	Sniffing the ground or of an item. Distance less than 5 cm.
	Drag	state	Dragging of the hind legs over the ground (leaving marks) followed by spray urinating.
	Spray urinate	instant	bull or females in oestrus lift their tail and spray their urine in a straight jet.
	Urinate	state	Urination, also called micturition, is the movement of urine from the urinary bladder through the urethra to the outside of the body. The tail is elevated and hind legs are spread slightly laterally.
	Defecate	state	The tail (of both sexes) is elevated during defecation.
	Overmarking	state	Placing dung above the introduced sample.

Table 2.4. continued

Fighting	Attack	state	With head lowered and ears erect, rhino growls and attacks with its horn.
	Feint	state	Conspecific animal approached. Head is lowered. Often additional grunting. If conspecific animal doesn't retreat attack will follow.
	Supplant	state	Approach of one rhino causes another to move away, without overt aggression.
	Kick	instant	One or both legs thrust forcefully backward toward another rhino.]
Interaction	Smell excrement	state	Sniffing of urine or faeces. Distance less than 5cm. Sniffing is performed during air intake with the nostrils.
	Testing	state	Animal smears the sample by dragging its upper lip in the sample.
	Flehming	state	Lip is curled dorsally and the head and neck are often outstretched and elevated. Accompanied by inhalation and exhalation.
	Smell animal	state	Sniffing a conspecific animal. Distance less than 5cm.
	Courtship	state	Extensive smelling of each others head, nose and body
Other	Rub horn	state	Scraping the horn against the ground or an object.
	Rub body	state	Rasping part of the body on an object.
	Rub head	state	Scratching head on an object.
	Rolling	instant	Rolling on the ground. The rhino may stay on one side or roll from one side to the other with legs off the ground. It may prop itself up on its forelegs as if to begin standing but then rub its sternum on the ground. The bout ends with the rhino propping itself up with its forelegs, moving the hind limbs under the body and using the hindquarters to push itself up.
	Chew	state	Open and close mouth against an object
	Squeak	instant	Squealing or whining.
	Snort	instant	Short forceful exhalations from nostrils. Can be an alarm call when head is elevated.
	Grunt	instant	Low pitched vocalization.

The following categories were initially considered but did not play any role during the observations: bite thread, kick thread, chase, mating, burrow, sitting, licking, jump, shying, touch, twitching, tailing, bite, fleeing, excite call.

2.7. Behaviour data collection

EthoLog (Ottoni, 2000) was used for real-time recording (on a laptop) when observing behaviour. Key codes (Appendix 1) for predefined behavioural categories were typed and EthoLog registered their sequence and timing and saved all information for subsequent data analyses. Data from an observation trial (15 minutes) were saved in two files: a report file and a log file, the filenames of which generally contain date, observed subject, and trial number. Thus "1511MP05" is the name of the EthoLog file recorded on 15 November 2005 while rhino Mapenzi was observed during the fifth trial on that day. For various reasons modifications of this naming convention occurred.

Each report file (ending .REP) contains

- File name
- Categories file used
- Transcription date and time
- Number of categories
- Session length
- Notes
- Number of state events (SE)
- Number of instantaneous events (IE)
- Summary statistics of category events with number of categories, duration of SE(s), number of SE(s), mean duration of SE(s), and number of IE(s)

Each log File (ending .LOG) contains

- File name
- State events sequence with number of category, category code name, start time (s), duration (s), end (s), and label
- Instantaneous events sequence with number of category, category code name, start time (s), and label

For the 13 tests with different sample types, 79 observation sessions were performed each session with 9 to 15 trials. A total of 957 report and 957 log files (Appendix 2 gives the EthoLog filenames) were generated. Six log files could not be used, because their data were corrupted.

2.8. Position data

At the beginning and the end of each session as well as in between the trials, positions of and distances between all subjects were recorded. The rhino enclosure was divided in eight sections and the number of the section was noted in which a subject was located. Distances in body length (ca. 4 m) were estimated between each two subjects. Note that the positions and distances were recorded only once every 20 min, i.e. any movement to other sections in between these trials was not recorded.

2.9. Environmental conditions

Temperature, humidity and other relevant environmental conditions were recorded each observation day at 9:00 AM and12:00 noon.

The following weather data were obtained from the nearby Christchurch airport (ca. 5 km distance from Orana Park):

– Temperature (°C)

– Relative Humidity (%)

- Clouds (*/8)
- Wind (km/h)
- Wind Direction in degrees
- Wind Direction on the compass
- Barometric Pressure (hPa)

2.10. Chemical analysis of faecal material

Three chemical constituents of faeces are considered relevant to this study:

1. Olfactory volatiles or pheromones in faeces samples are the transmitters of messages to other rhinos (Bradshaw, 2003). Therefore, the number of different volatiles and their nature was analysed.

2. Testosterone is the principal male sex androgen. It is present in highest levels in the faeces of the most dominant bulls (Kretzschmar, 2002).

3. The Major Histocompatibility Complex (MHC) is a large genomic region or gene family found in most vertebrates. It contains many genes with important immune system roles. Recent work suggests that sibling discrimination by vertebrates may be based on major histocompatibility complex (MHC) genes that affect the composition of pheromones and/or urine (Olsen et al., 1998). MHC genes control immunological self/non-self recognition (Penn & Potts, 1999). If white rhinos prefer mates with a dissimilar MHC, this mating preference might result in MHC heterozygous offspring that may have enhanced immunocompetence. Due to time constraints, however, it was not feasible in this study to perform the necessary tests to determine this constituent.

In the following paragraphs analysis of the first two constituents is described.

2.10.1. Analysis of olfactory volatiles

The analysis of olfactory components was performed using gas chromatography mass spectrometry (GC-MS). This system consists of two separate units; the gas chromatograph (GC) that separates volatile mixtures, and the mass spectrometer (MS). The mass spectrometer fragments and ionizes the gaseous sample molecules and analyses and detects the mass-separated ions. A computer system provided data processing during and after collection (Smith & Busch, 1999). Analyses were done on the headspace volatiles (Headspace is the vapour mixture trapped above a solid or a liquid in a sealed vessel). Headspace volatiles were injected into the GC and the gas was carried by inert helium gas through the GC column. The column is a 30-mlong thin tube with a special polymer coating on the inside. Volatile molecules are separated because highly volatile species travel quicker than species of low volatility. Following the GC the gas enters the ion source of the mass spectrometer where the molecules are bombarded with electrons, causing the chemicals to fragment into positively charged ions. The ions are accelerated and fly through an electromagnetic mass filter where they are separated according to their molecular mass. Finally, the detector measures the number of ions of a specific mass. These data are sent to a computer where they are stored and a mass spectrum is displayed.

A mass spectrum is a diagram of the number of ions of different masses that passed through the mass filter plotted against mass. Particular parent molecules have a specific fragment pattern of mass lines. Chemical identification was completed by computer comparison of the mass spectrum of an unknown compound to mass spectra gathered into a database, in this case the Wiley's Database. This database is a compilation of peer-reviewed mass spectra gathered from numerous GC-MS. From the identification of chemicals, pheromone patterns and differences within donor samples were established.

For the analysis a small amount of faecal material (ca 5 ml) was put in a vial. The vial was then topped with a silicon top and clamped with an aluminium ring. The sample was heated to 40°C for at least an hour prior to analysis in a temperature controlled water bath. A one ml aliquot of headspace was withdrawn for injection.

The samples from ten donors were analysed in a Kratos MS80RFA high resolution mass spectrometer coupled to a Carlo Erba MFC500 capillary gas chromatograph. The GC column was

a Zebron (phenomenex) ZB-WAX GC column (30m x 0.25mm internal diameter with phase thickness 0.25 μm).

The GC temperature was programmed with an initial temperature of 30°C, held for 5min after injection, and increased by five °C/min to 200°C which was held for a further 10 min. The injector was held at 160°C.

The purpose of the initial analysis was to detect olfactory differences between the samples and not to identify the specific compounds. Therefore, the temporal development of key mass lines (at 57, 60, 71, 85, 88, 91, 93, 98, 100, and 136 dalton) was monitored. These masses were selected because they indicate major fragmentation products of the olfactory components and they were present at all retention times when the gas chromatogram displayed significant peaks. Complete mass spectra and their potential identifications were obtained later for those components which were characteristic of samples that caused the strongest reaction by the subjects during the different tests.

2.10.2. Testosterone concentration

The hormone levels of the donors were determined from the collected samples. In addition, faeces samples of the male subject were collected during the observation.

Testosterone measurements of faecal sample were done by Dr. John Lewis (Canterbury Health Laboratories). Forty-nine different samples (several samples from five male donors, peat, and heated samples) were analysed for testosterone concentrations. For the measurement, each individual sample was divided into three sub samples which were analysed in separate runs. Runs 1 and 3 included control measurements each consisting of five individual samples of different known testosterone levels. Runs 1 and 2 refer to testosterone and its metabolites, while Run 3 refers to testosterone alone.

For the measurements of the concentrations of testosterone and its metabolites, 200 mg of dried faeces were extracted with five ml of 80% methanol overnight by rolling gently in sealed vials. The clarified extract was then diluted (1:10) in testosterone assay buffer and analysed by enzyme-linked immunosorbent assay (Elder & Lewis, 1995). For testosterone 200mg of freeze dried faeces was dispersed in two ml water and then extracted with five ml diethyl ether. The dried

extract was dispersed in 1600 µl of testosterone assay buffer and testosterone analysed by ELISA.

Each sample was measured twice and the mean value calculated. At least two concentration values were necessary to compute the mean concentration for a specific sample type.

2.11. Data analysis and statistical tests

A key task of this thesis was to translate as complete as possible the subjects' behaviour, positions, and environmental conditions into machine readable form. Thereby, a computer image of the behavioural states and supporting information was obtained which was used to visualize the data, to correlate various behavioural and other aspects and to apply statistical tests.

Behavioural data contained in EthoLog output files, both .LOG and .REP file are pure ASCII files that were read into a Microsoft Excel spreadsheet program for manipulation and initial review of the data. Similarly position, weather, GC-MS, and testosterone data were initially recorded in Excel files.

In order to merge data from the various sources they were individually read into a Microsoft Access data base. The merged data products were then exported back to Excel for generating diagrams. However, since the capabilities for displaying the data in various formats are very limited and in many cases inadequate it was decided to move data analysis to IDL (Version 5.1) from Research Systems Inc. IDL is an interactive data language which supports easy plotting of data.

All EthoLog output files were directly read in by an adapted IDL program. All other data were read out from Excel into comma-separated ASCII files and read in by the IDL program. All diagrams shown in this thesis and all statistical analyses were done in IDL.

In this thesis commonly mean values, variances, and standard deviations SD are calculated. The mean value of a group of values is the sum of all values of a group divided by the number n of elements of this group. The variance is the sum of the squared difference between the mean value and the individual values. This sum is then divided by (n-1). The standard deviation is the square root of the variance.

The purpose of nonparametric statistical tests in this thesis is to test whether different groups of values (e.g. the behavioural reactions to different sample types) have significant different means. This test is done by the Wilcoxon Rank-Sum (WRS test) when data from two independent populations are compared and by the Kruskal-Wallis H-Test (KWH test) when three ore more populations are compared (Bortz, 1999; Lee et al., 2000). Both Tests are implemented in IDL.

The WRS function tests whether two sample populations, X and Y, have different means. X and Y may have different numbers of elements. The result is a two-element vector containing the nearly-normal test statistic Z and the one-tailed probability p of obtaining a value of Z or greater. The hypothesis that two sample populations have the same mean of distribution is rejected if they differ with statistical significance ($p < 0.05$).This type of test is often also referred to as the "Mann-Whitney U-Test". When data was used for multiple comparisons a Bonferroni adjustment was used.

The KWH test function tests whether three or more sample populations have the same mean. The populations may be of equal or unequal lengths. The result is a two-element vector containing the test statistic H and the one-tailed probability p of obtaining a value of H or greater from a Chi-square distribution. The hypothesis that three or more sample populations have the same mean of distribution is rejected if two or more populations differ with statistical significance ($p < 0.05$).

Two ways of fitting measurement data x and y to theoretical curves are used: 1. Fit to a linear model $y = a + b \cdot x$, and 2. Fit to an arbitrary function $y = f(x)$. A linear fit procedure implemented in IDL is used to calculate the constants a and b and their errors by minimizing the chi square error statistic. The uncertainties of the measurement data are used as weighting factors in the fitting procedure. The weights are defined as the reciprocal squares of individual standard deviations of the measurements. The fit to a user defined curve is also performed by an IDL routine that uses a gradient-expansion algorithm to compute a non-linear least squares fit. Iterations are performed until the chi square changes by less than 10^{-3}.

Chapter 3

3. Results

3.1. Behaviour and Positions

3.1.1. Daily activities

I present two examples of daily activities in order to illustrate what the raw data look like. This is done in the form of graphical activity diagrams derived from the Etholog data (see appendix).

September 30, 2005, was a typical low-activity day when a control sample was presented to the subjects (Fig. 3.1). Behaviour data for each subject are represented by a different colour. Diamonds mark the behaviour of all animals observed in scan sampling mode at 20-min intervals. In between, a specific individual animal was observed for 15 min in focal animal mode. Behaviour state are marked by triangles (which show the start of a specific behaviour) and attached bars which indicate the duration of this event. Instantaneous events are marked by crosses.

September 30 was a partly cloudy spring day with north-easterly winds, and a noon temperature of 18°C. Most of the time all animals were just feeding and, towards the end of the observation session, they were lying down and resting.

The only instantaneous events recorded during this session were spray urinating by Cyrano, apparently triggered by scanning and smelling the ground. This was accompanied by more walking than usual. Spray urinating occurred in about three bursts in rapid succession (with a bout length of only a few seconds). Generally, the male displayed more varied behaviour than the females.

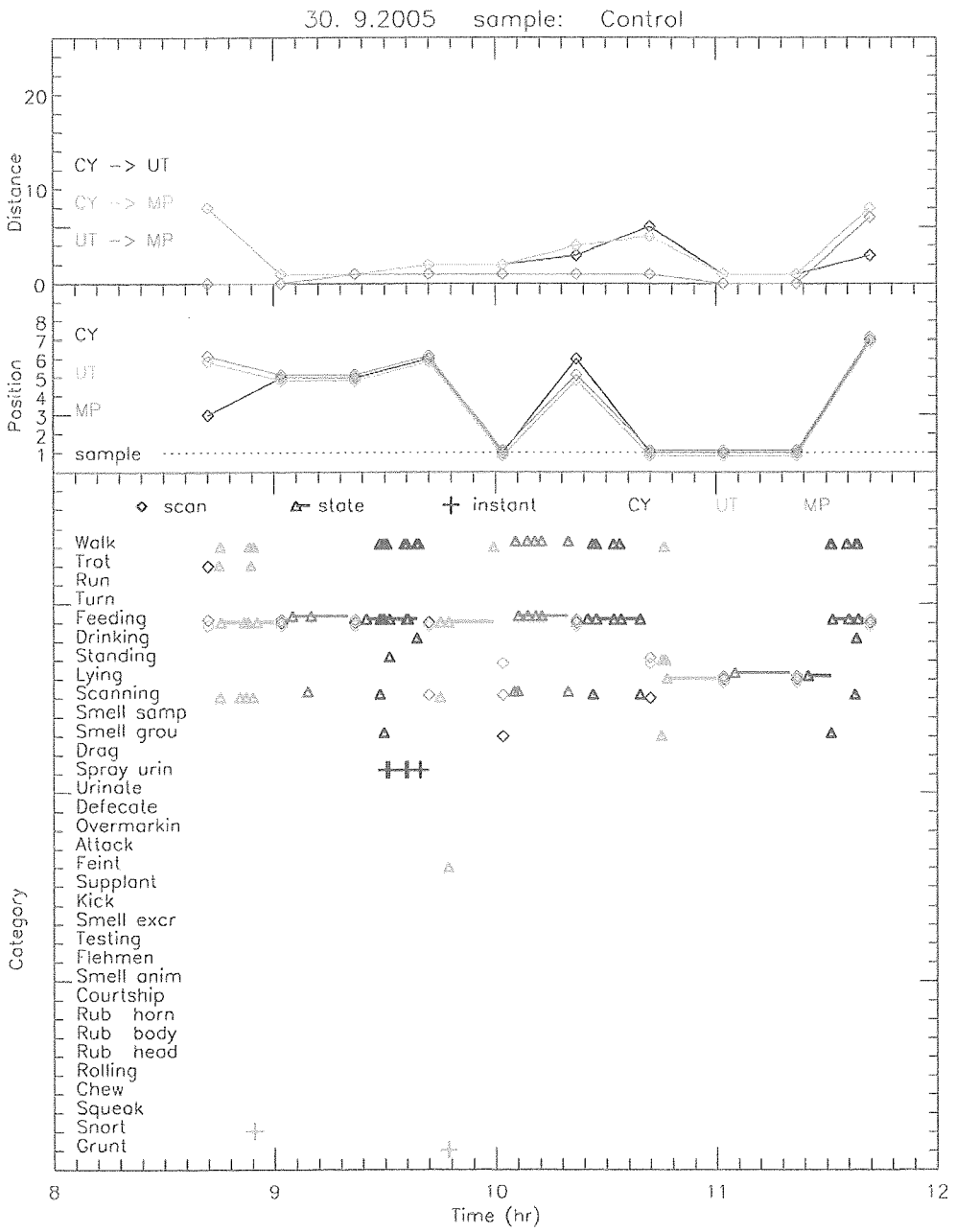

Figure 3.1. Lower panel: Behavioural activity on 30 September 2005. Behaviour observations in the scan sampling mode are identified by diamonds. Behaviour state events observed in focal animal mode are marked by triangles (start of a specific behaviour) and attached bars indicate the duration of this behaviour state. Instant events are marked by crosses. Colours represent different animals. Upper panels: Positions of and distances between animals on 30 September 2005. Estimated distances between any two animals in animal lengths (ca. 4 m).

Rhinos moved slowly about in the enclosure during observation periods, but they stayed close together (e.g., see Fig. 3.1). Note that the positions were recorded only once every 20 min (any movements to other sections in between these intervals were not recorded).

An observation session started after all animals entered the enclosure from their respective night shelters. Utani usually walked in first, straight down to the piles of hay laid-out by the keeper (section 5-7). The oryx gave way as soon a rhino came closer than about 10 m. On rainy days the hay was presented covered by trees in section 2. Mapenzi's routine was first to scan the enclosure and then to follow Utani. Cyrano was released last. Often he trotted right away to the food and joined the females. It was not unusual for him to spray urine over his food before moving on to another pile. After about 45 min, the rhinos left the presented hay and grazed through the enclosure. It was on these occasions that the rhinos were most likely to discover samples that were present. Generally, the females stayed close together (within 3 body lengths of each other), while Cyrano was near, but sometimes they wandered off, especially when samples from male donors were presented. Mapenzi seemed to scan more than the other rhinos. Visitors, the regularly passing zoo shuttle, air planes or gun shots from a nearby shooting range caused no apparent response. Around noon the females occasionally rested in the sand pit (sector 1) for roughly 20 to 40 min. During the three-hour observation session, they generally moved through most of the enclosure, visiting most sections at least once (Fig. 3.2).

More territorial activity (scanning, smelling the sample, smelling the ground, dragging and, most obviously, spray urinating) was displayed by Cyrano on 16 November 2005 (Fig. 3.3.) when the dung sample from the hidden Christchurch bull was exposed. Note that, prior to 14 November 2005, only one animal was observed during each 15-min trial, so information on the behaviour of the other animals was not recorded. After 14 November 2005 the observation sequence was changed so that there were parallel observations of the male, Cyrano, and the females.

November 16 was an overcast day with strong cold winds from the west and south. Noon temperature was 13°C. At about 9:30 A.M. Cyrano became agitated and repeatedly smelled the ground and started heavily spray urinating while he moved close to the sample site. Bursts of spray urinating were accompanied by scanning, smelling the ground, smelling the sample, walking, and even trotting. Over the course of about an hour he slowly calmed down and started

feeding again. Towards the end of the session, Cyrano and Mapenzi began fighting, first by supplanting and then attacking each other.

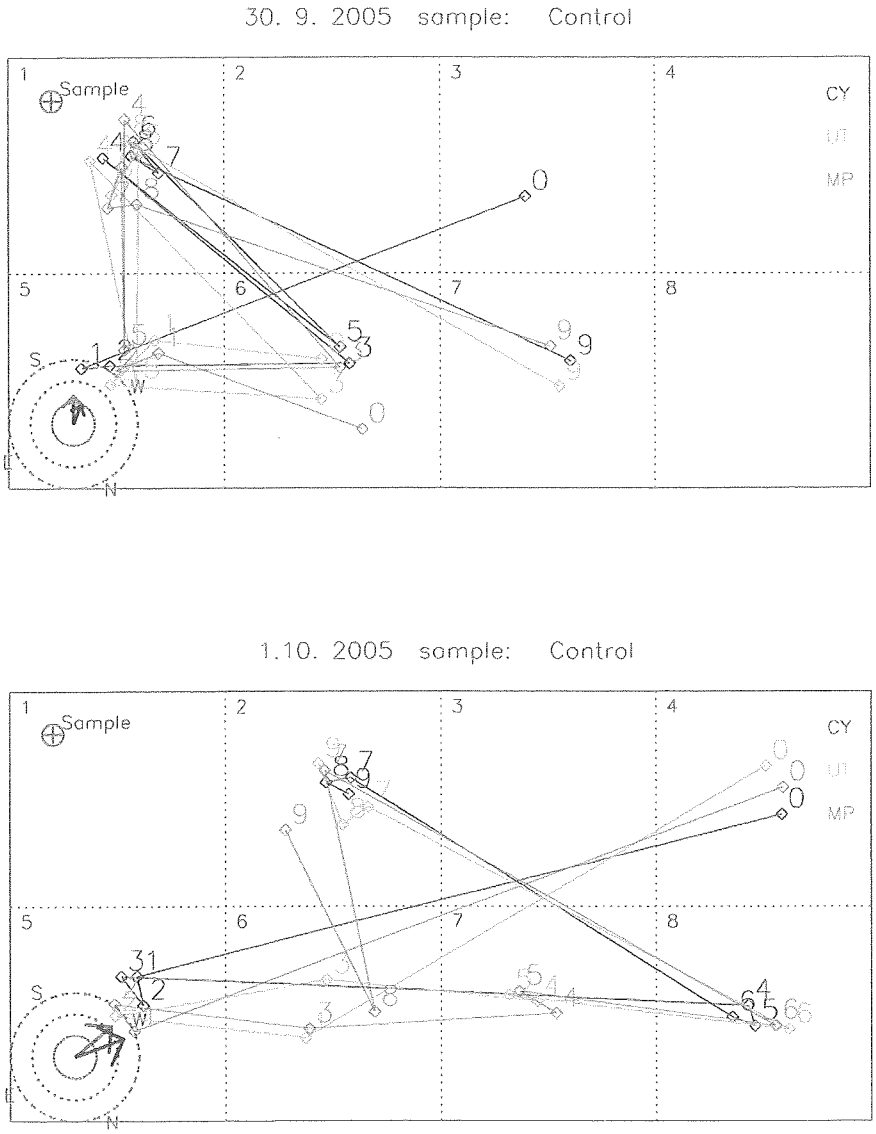

Figure 3.2. Movement of the animals during the observation session on 30 September and 1 October 2005 throughout the Orana rhino enclosure. Rectangle is a schematic representation of the Orana rhino enclosure divided into 8 sections. Positions of an animal (marked by diamonds of a specific colour) at times 15 minutes apart are indicated (for display purposes positions are randomly scattered around the section centre). Numbers attached to the positions indicate the sequence (0 to 9) of positions during a day's session. The sample position is in section 1. In the lower left corner (section 5) the wind direction is indicated by a compass rose, both for the beginning (9:00, black arrow) and end (12:00, red arrow) of the daily observation sessions. Dotted circles correspond to wind speeds of 10, 20, and 30 km/h, respectively.

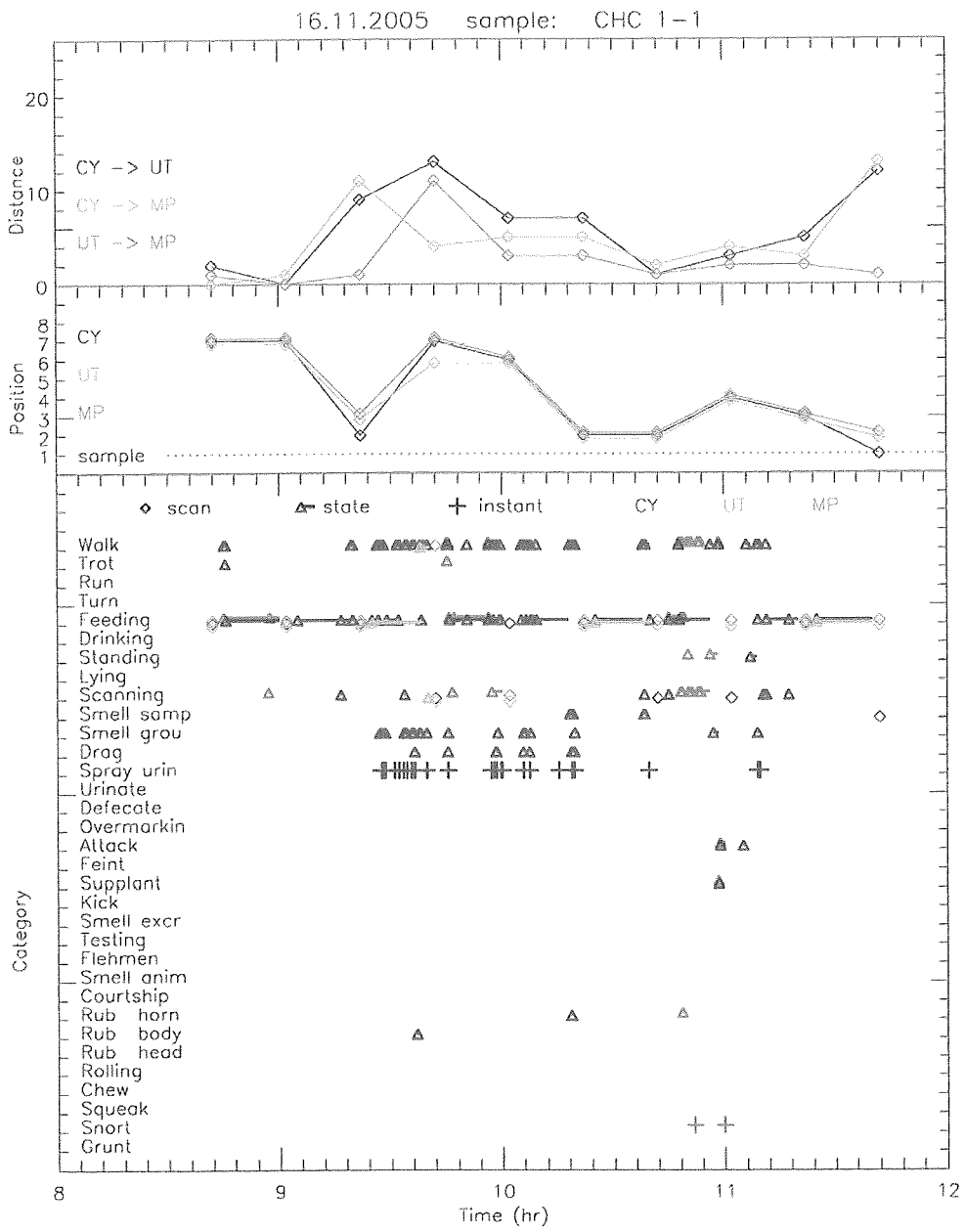

Figure 3.3. Activity diagram of 16 November 2005. Behavioural activity (lower panel). Positions of and distances between animals (upper panels). For more information see Figure 3.1.

The next day Cyrano stayed alarmed (i.e., he frequently scanned, smelled the ground, smelled the sample, walked and spray urinated). During all sessions, when the sample from the hidden Christchurch bull was presented, Cyrano showed elevated territorial activity. Other dung samples also triggered similar behavioural activity.

3.1.2. Frequency of occurrence of different states and behaviour categories

From quantitative analysis of behaviour states (Fig. 3.4), for September 30 2005, feeding, walking, scanning, and spray urinating (only Cyrano, ten times) were the most frequent events. However, feeding occupied about 80% of the time of all animals. On 16 November 2005 (Fig. 3.5.) the number of spray urinating events increased by a factor of eight! The frequency of walking also increased, suggesting that the exposed dung sample from the hidden Christchurch bull caused this increase.

By far the most frequent behaviour categories were walking, feeding, and spray urinating (Figs. 3.6, 3.7; Table 3.1). Although Cyrano's frequency of activities in these categories dominated those of the females by at least a factor of two, the total time spent feeding was comparable for all animals.

Flehmen (Grzimek, 1990; Pflumm, 1996) is an important mechanism for the detection of sexual scents used by many mammals. It is performed with the chemo receptor in the vomeronasal organ. It was only observed on 44 occasions, which represents less than once per observation session. No significant enhancement of the frequency or duration of flehmen was observed during any test. Cyrano performed flehmen only after tasting his females' urine. Since urinating by the females did not change in response to any sample, there was no change in flehmen observed as well.

Next, I examined individual behaviour categories and their variations during the different observation sessions. Of all behavioural categories, the results for spray urinating presented the strongest and clear-cut response to the test samples. The mean frequency of spray urinating by Cyrano during the 79 observation sessions (Fig. 3.8) varied from no event to about 12 events per 15-min trial. Means were derived over a test period with one sample type. The mean values ranged from about one to more than six events per 15-min trial. The high values clustered for tests with dung samples from male donor animals from Christchurch (CHC) and Auckland (AUC A and AUC B). The lowest mean values were observed for sample types from the Hamilton juveniles (HAM b and HAM c), the Hamilton female (HAM Z), and the peat and control sample types.

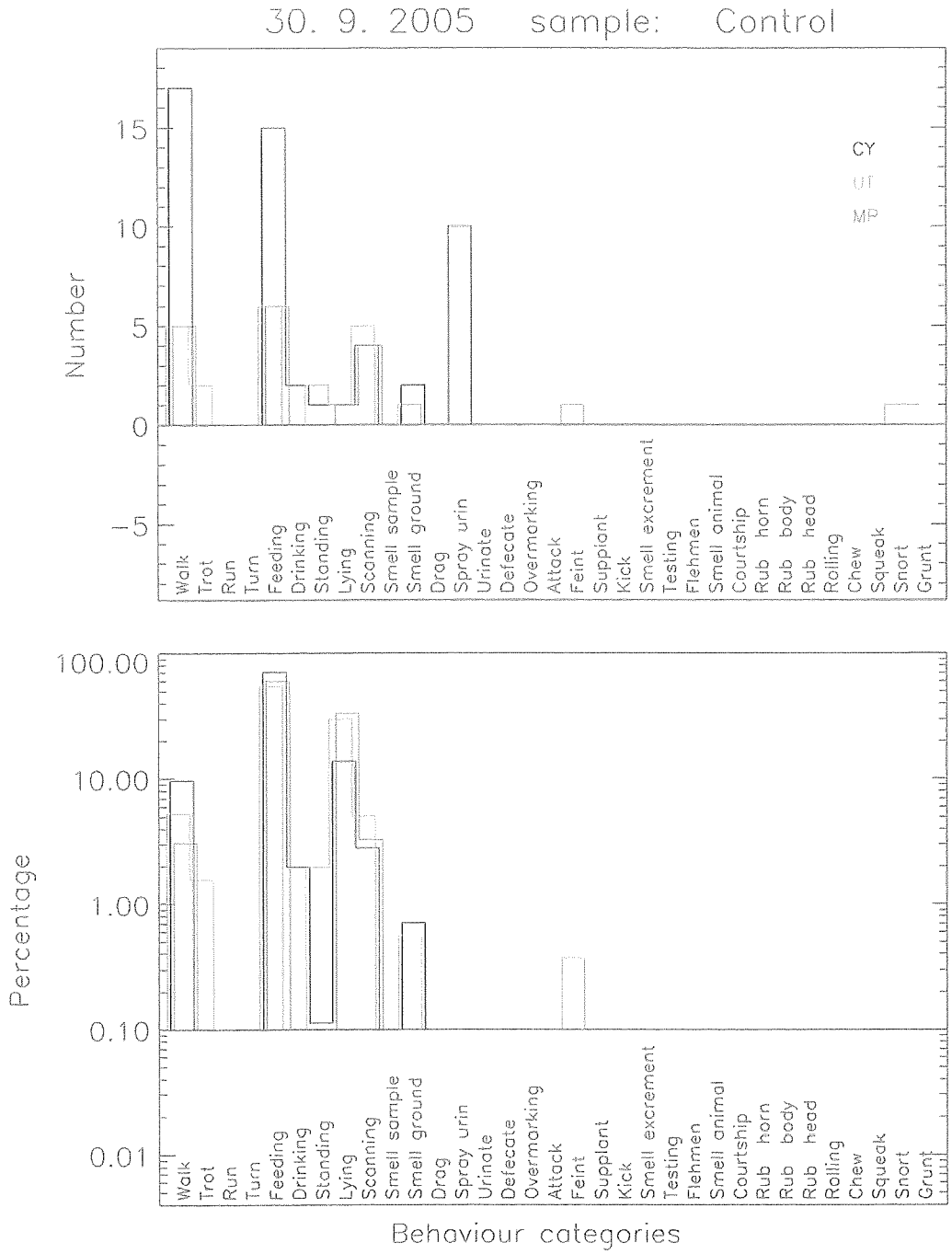

Figure 3.4. Behaviour statistics on 30 September 2005 (low spray urinating activity): number of occurrences (upper panel) and percentage of time (state events, lower panel). Animals are colour coded.

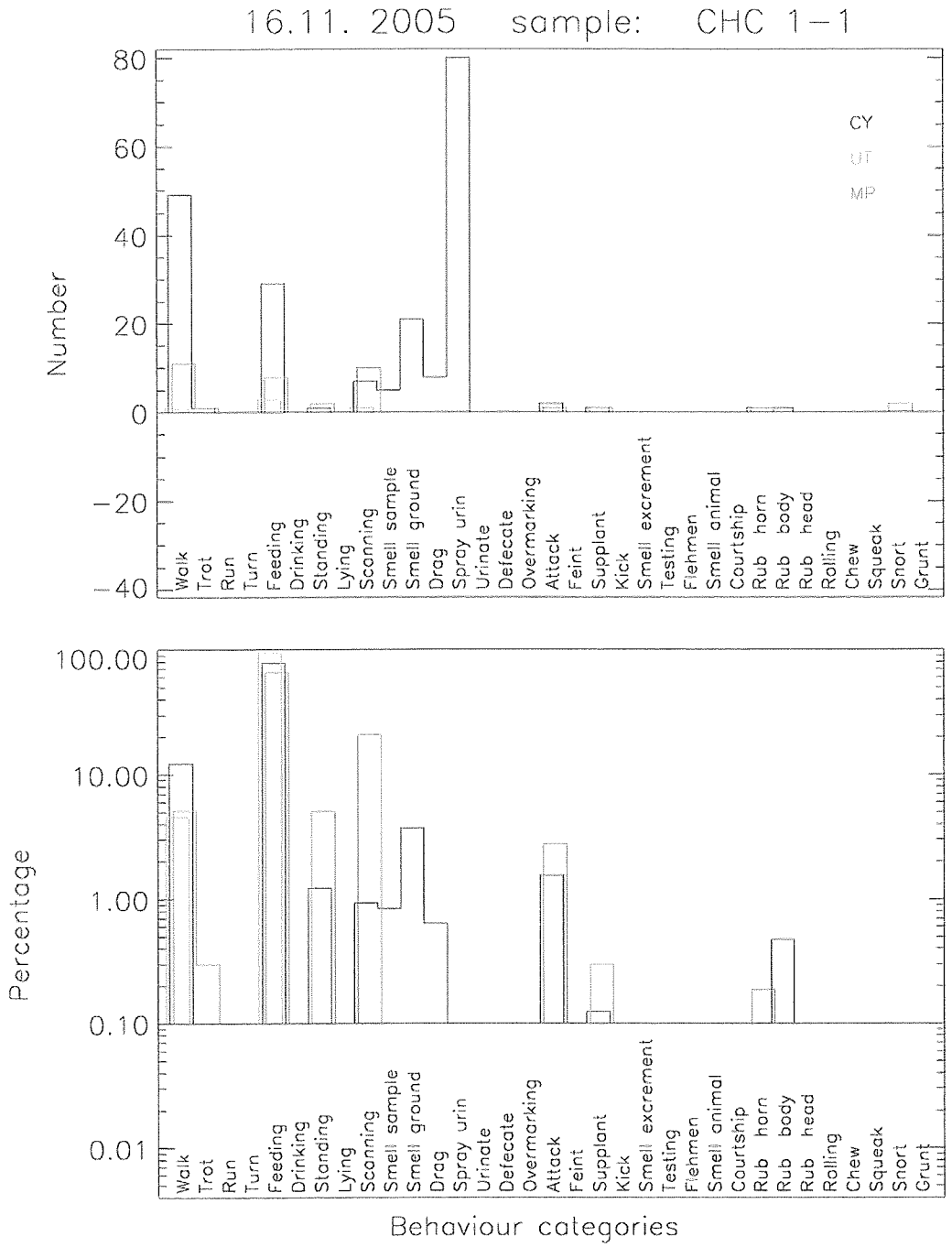

Figure 3.5. Behaviour statistics on 16 November 2005 (high spray urinating activity): number of occurrences (upper panel) and percentage of time (state events, lower panel).. Animals are colour coded.

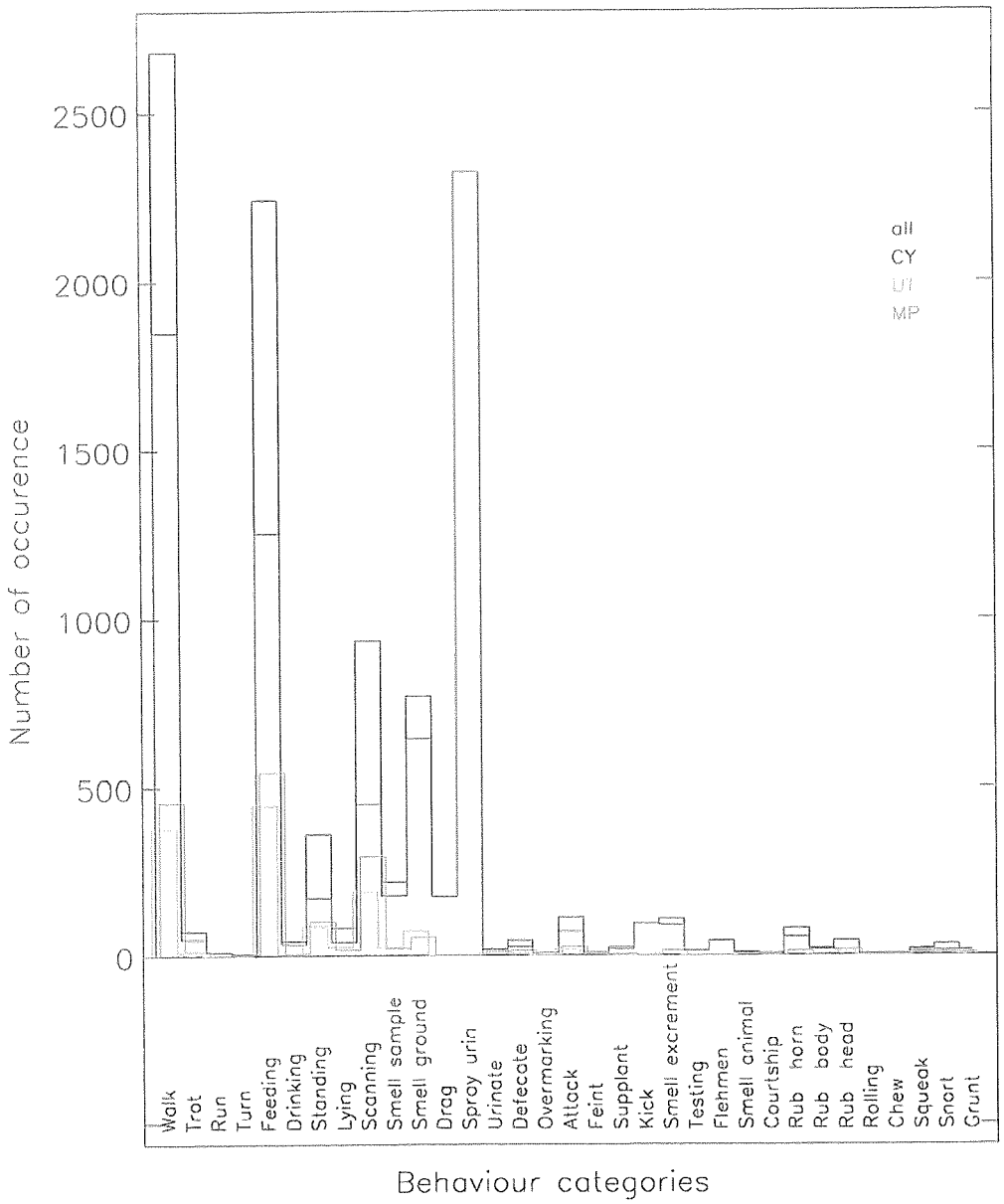

Figure 3.6. Behaviour statistics: number of occurrences of all categories. Animals are colour coded.

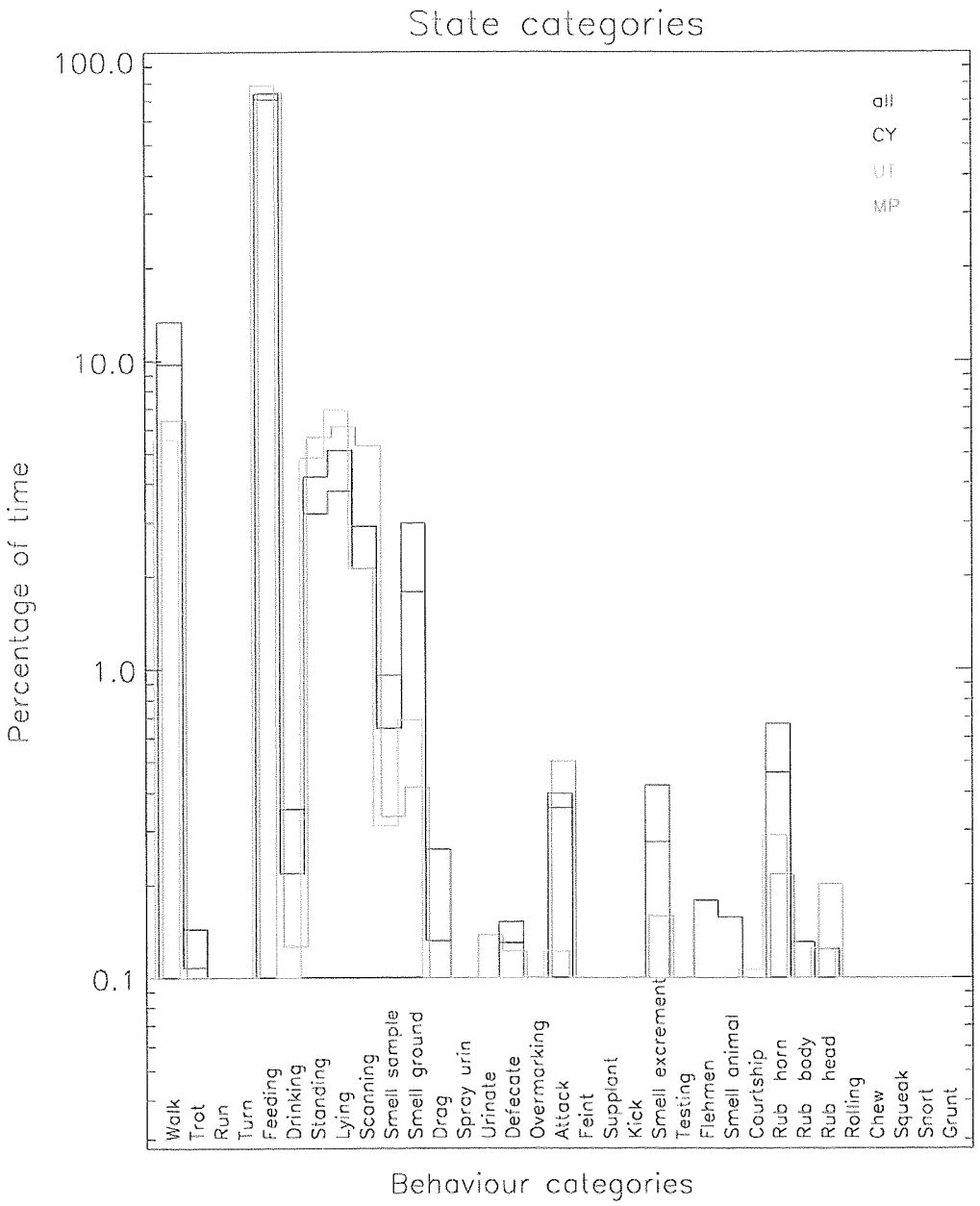

Figure 3.7. Behaviour statistics: percentage of time for all state categories. Animals are colour coded.

Table 3.1. Frequency of behaviour events. Data cover all experimental observations in this study. Total number of occurrences listed, with percentage of time for each state or event in parentheses)

	Number of occurrences (percentage of time)		
Category	**Cyrano**	**Mapenzi**	**Utani**
Walk	1847 (13.38%)	379 (5.57%)	454 (6.42%)
Trot	49 (0.14%)	9 (0.08%)	15 (0.06%)
Run	11 (0.09%)	0 (0.00%)	0 (0.00%)
Turn	1 (NA)	0 (NA)	4 (NA)
Feeding	1254 (70.39%)	446 (78.45%)	542 (74.11%)
Drinking	34 (0.35%)	3 (0.03%)	5 (0.13%)
Standing	171 (3.20%)	89 (4.84%)	101 (5.66%)
Lying	41 (3.76%)	24 (6.95%)	17 (6.13%)
Scanning	450 (2.12%)	191 (2.13%)	293 (5.32%)
Smell sample	177 (0.95%)	23 (0.31%)	20 (0.33%)
Smell ground	644 (2.98%)	74 (0.68%)	53 (0.41%)
Drag	175 (0.26%)	0 (0.00%)	0 (0.00%)
Spray urinating	2324 (NA)	0 (NA)	0 (NA)
Urinate	1 (0.01%)	6 (0.10%)	11 (0.14%)
Defecate	25 (0.15%)	7 (0.09%)	13 (0.12%)
Overmarking	5 (0.03%)	0 (0.00%)	0 (0.00%)
Attack	71 (0.40%)	17 (0.12%)	25 (0.50%)
Feint	3 (0.01%)	2 (0.01%)	3 (0.02%)
Supplant	18 (0.03%)	1 (0.00%)	4 (0.01%)
Kick	95 (0.00%)	0 (0.00%)	0 (0.00%)
Smell excrement	90 (0.42%)	5 (0.09%)	13 (0.16%)
Testing	15 (0.02%)	0 (0.00%)	0 (0.00%)
Flehmen	44 (0.18%)	0 (0.00%)	0 (0.00%)
Smell animal	7 (0.16%)	1 (0.01%)	1 (0.00%)
Courtship	3 (0.06%)	2 (0.11%)	0 (0.00%)
Rub horn	55 (0.66%)	11 (0.29%)	13 (0.22%)
Rub body	17 (0.13%)	1 (0.01%)	2 (0.06%)
Rub head	16 (0.09%)	12 (0.12%)	16 (0.20%)
Rolling	4 (0.00%)	0 (0.00%)	0 (0.00%)
Chew	4 (0.04%)	0 (0.00%)	0 (0.00%)
Squeak	10 (NA)	2 (NA)	5 (NA)
Snort	14 (NA)	9 (NA)	8 (NA)
Grunt	8 (NA)	1 (NA)	6 (NA)

NA: not applicable for instantaneous events

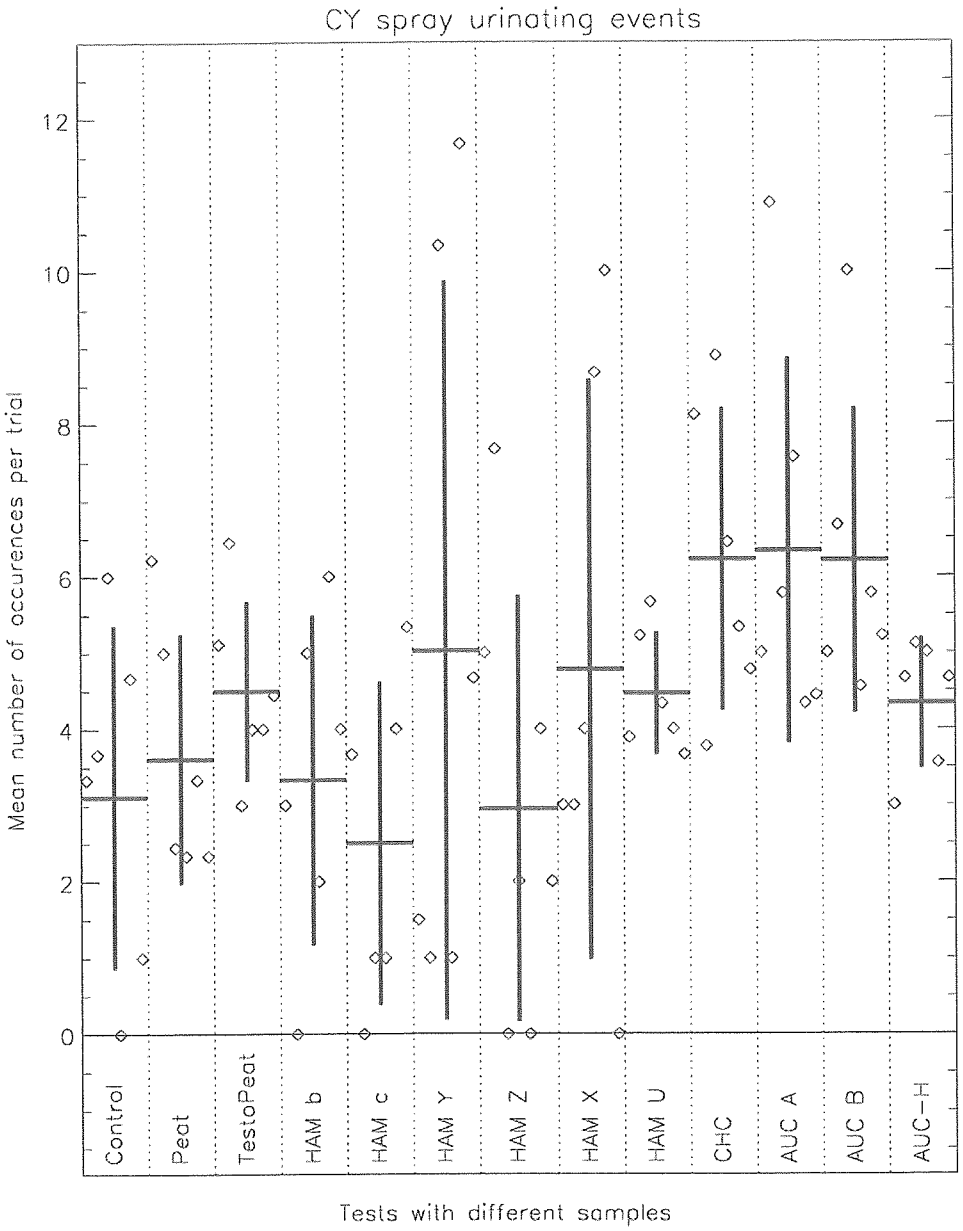

Figure 3.8. Frequency of spray urinating by Cyrano for all tests with different samples. Diamonds indicate means of individual observation sessions. Thick bars indicate the mean frequencies for specific tests (i.e. sample types).

The percentage of time spent feeding (Fig. 3.9.) during observation sessions varied from as low as about 30% to as high as about 98%, with means ranging from 60 to 90% during the different tests. The animals were walking from 5 to 20 per cent of their time (Fig. 3.10). Data from Cyrano, the male, were significantly different from data from females. For each sample type the Wilcoxon Rank-Sum test showed that the walking times of male and females differed with statistical significance ($p < 0.05$). Cyrano walked between 8 and 18% of the time, whereas females walked only 5 to 10% of the time.

An analysis of the distribution of the residence times showed that the animals spent about equal times in the middle and south-east sectors of the enclosure whereas they spend significantly less time in the north-west sectors (sectors 4 and 8, Fig. 3.11).

3.1.3. Habituation

In the presence of some samples, Cyrano's spray-urinating response was strong in tests with samples from the Auckland and Christchurch bulls AUC A and CHC. During sessions with other samples, only a few events of spray urinating were observed. However, during the tests with dung from males, many of these events were observed (Fig. 3.12). Spray urinating was accompanied by other activities, such as like scanning, smelling the sample, and smelling the ground. These bursts of activity followed roughly the same pattern: after sudden onset, spray urinating tapered off over a period of an hour. After that, Cyrano continued with his normal activity.

In order to get a more quantitative indication on the time scale of habituation, I added-up all time series of spray urinating events around the time when Cyrano smelled the sample the first time (Fig. 3.13). The frequency, f, of spray urinating events starting at time $t = 0$ is fit by an exponential function.

$$f = n_0 \cdot e^{-t/\tau}, \qquad (1)$$

where τ is the decay time constant and n_0 is the value at time t = 0 min. The decay time constant represents a standard time at which the frequency of events has decreased to 37% of the starting value n_0 at time $t = 0$. This parameter is useful for comparing the rapidity of habituation in different tests.

Decay time constants for different bursts ranged from 20 min to 100 min. Standard errors of the fit parameters were small. From session to session with the same sample typecno obvious systematic variations of the frequency of spray urinating events (c.f. Fig. 3.12) or habituation time constants was observed.

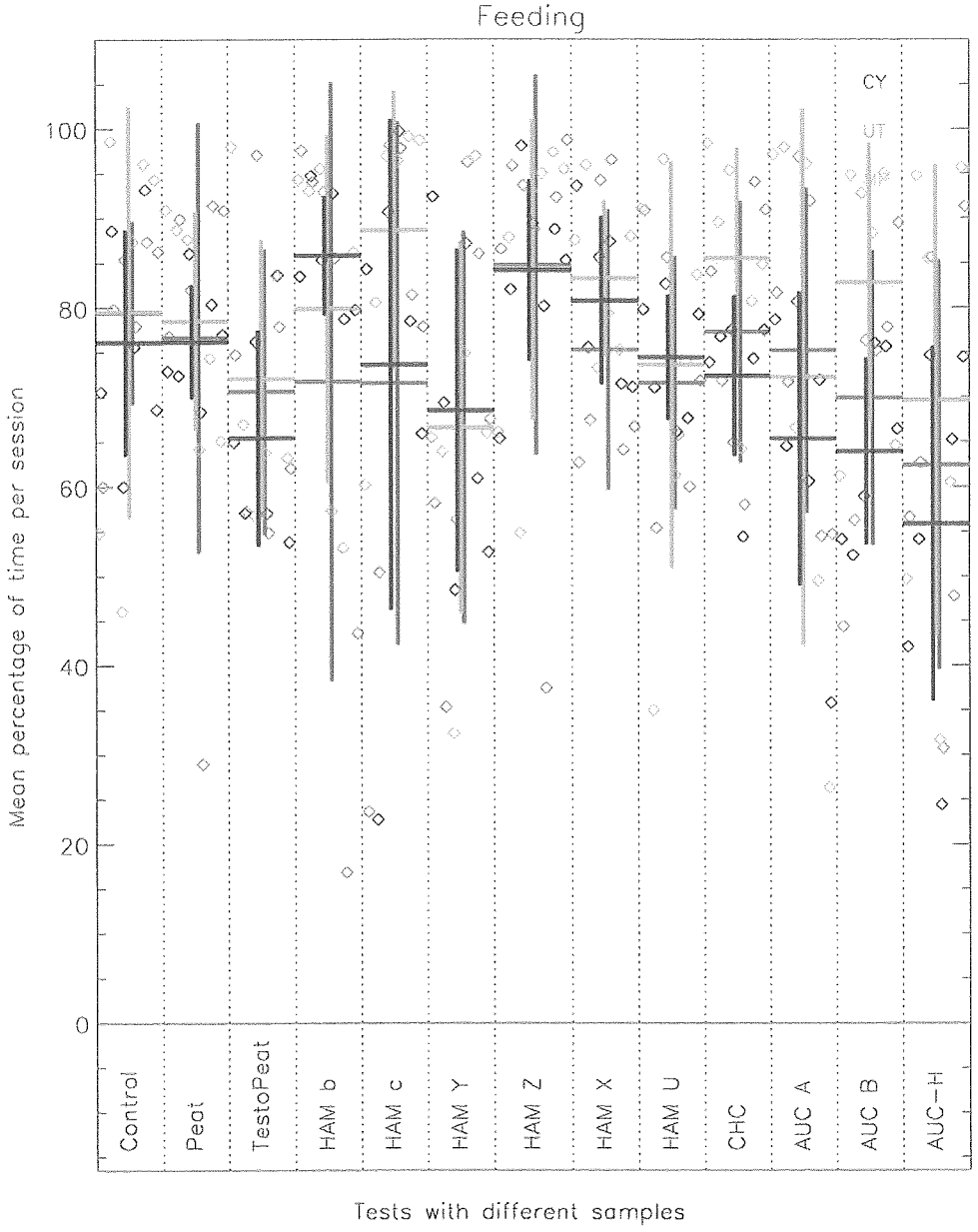

Figure 3.9. Percentage of feeding time for all tests with different samples. Diamonds indicate means of individual observation sessions. Thick cross indicates the mean frequencies for specific tests and animals (colour coded).

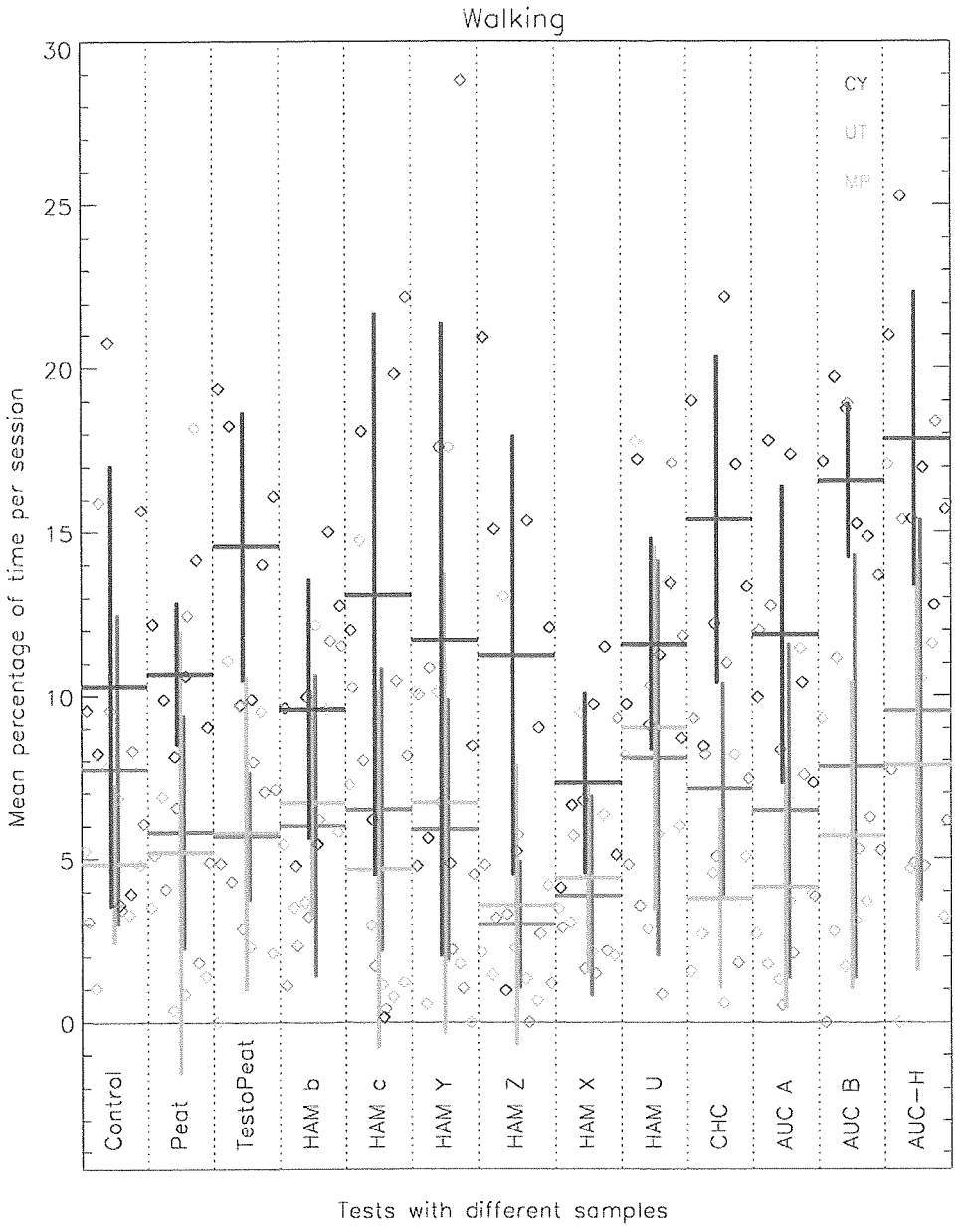

Figure 3.10. Percentage of walking time for all tests with different samples. Diamonds indicate means of individual observation sessions. Thick cross indicates the mean frequencies for specific tests and animals (colour coded).

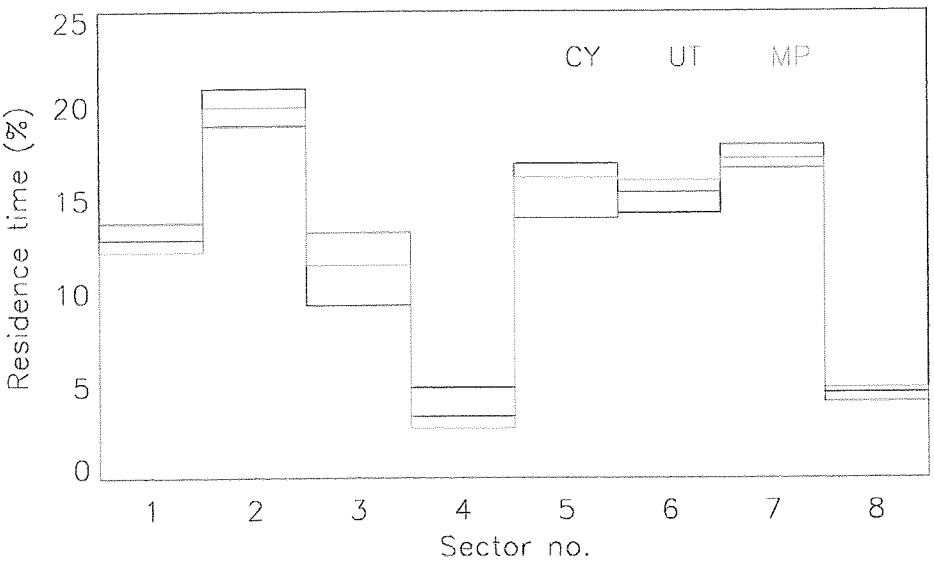

Figure 3.11 Residence time of the animals (colour coded) in the sectors of the enclosure.

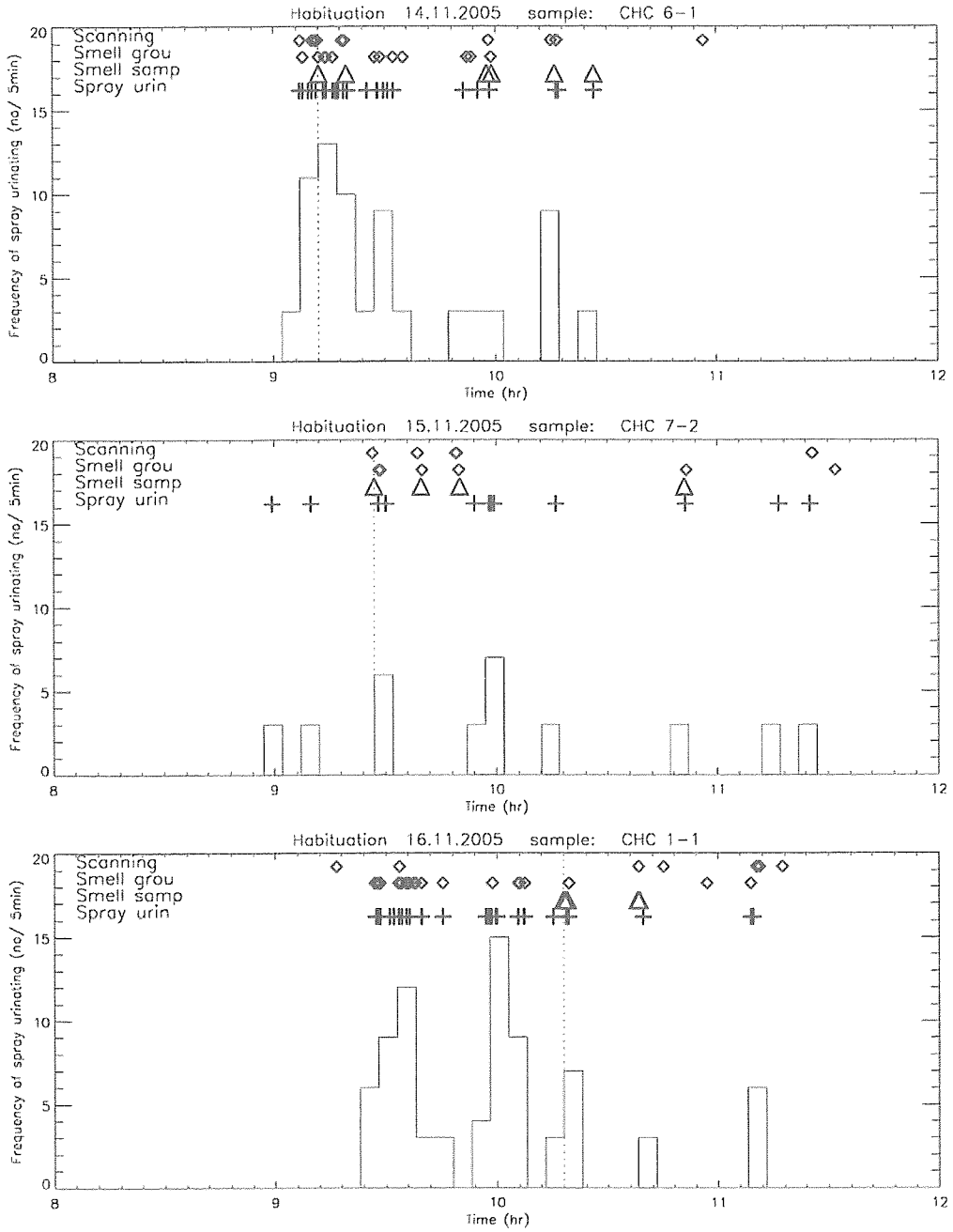

Figure 3.12. Frequency of spray urinating events during the observation sessions on November 14, 15, and 16, 2005. Top: Scanning, Smelling the ground, Smelling the sample, and Spray urinating events are indicated by diamonds, triangles and crosses, respectively.

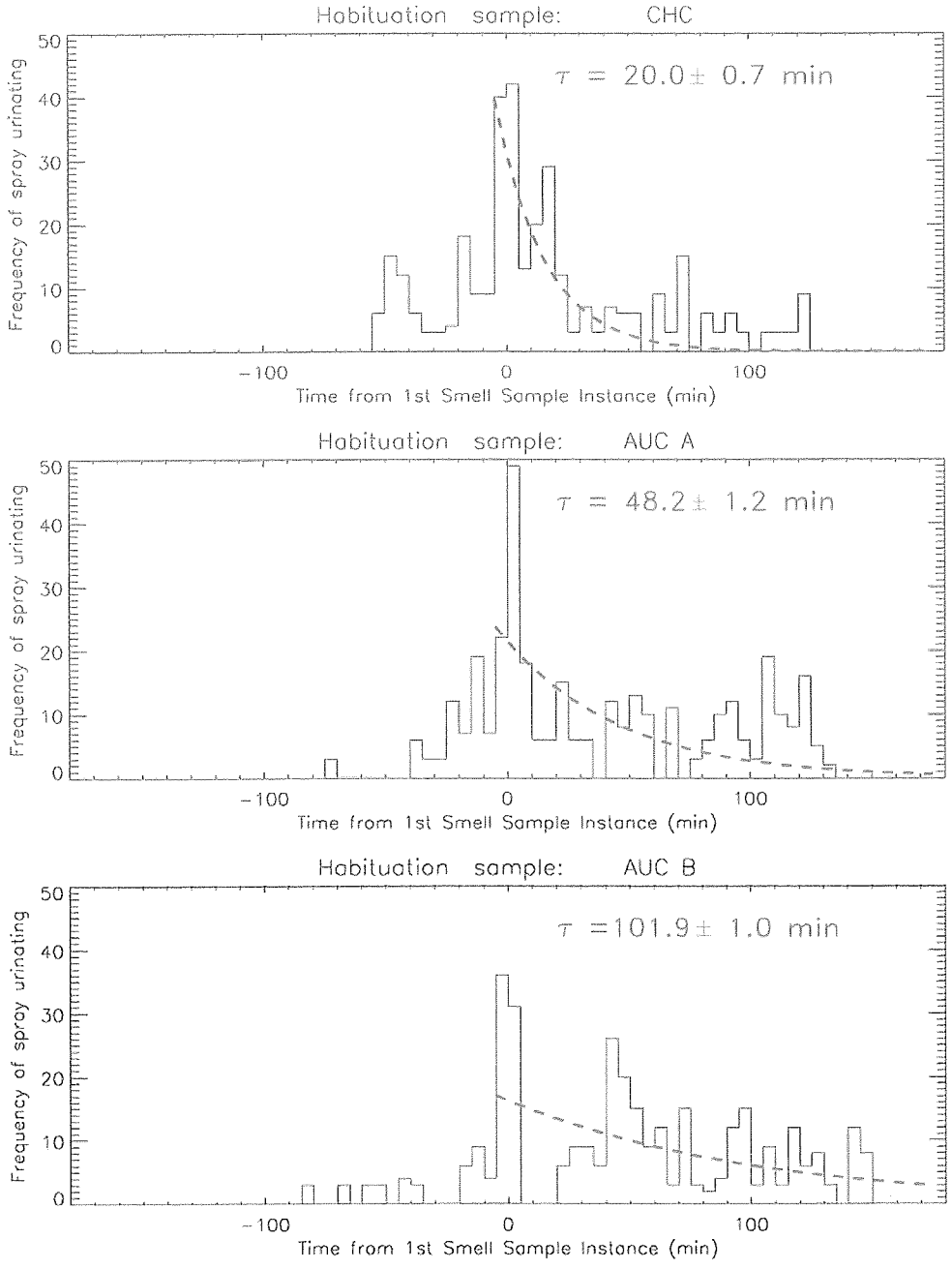

Figure 3.13. Habituation to samples presented to Cyrano from male rhinos CHC, AUC A and AUC B illustrating different rates of habituation. Histogram is distribution of spray urinating frequency around first smell sample event at time 0. Dashed curve represents the best fit of an exponential function (blue dashed line) of decay time constant τ (and standard deviation).

3.1.4. Spray urinating

To determine which samples triggered enhanced spray urinating, I compared the ensemble of records of spray urinating per trial for all sample types with that of the control measurements by applying the Wilcoxon Rank-Sum (WRS) test. This method tests the hypothesis that data of tests with various sample types do not differ from data of the control test ($p \geq 0.5$) or that they differ with statistical significance ($p < 0.05$).

The frequency of spray urinating in tests with different sample types spread from 2.5 to 6.3 events per trial (Table 3.2). Data from testing with sample types from the male donor animals AUC A, CHC, AUC B, and HAM U were statistically significant from data from the control tests.

Table 3.2. Occurrences of spray urinating (mean number per trial; standard deviation, SD). Z: test statistic from Wilcoxon Rank-Sum (WRS) test. P: probability from comparing distributions of control test with those of experimental tests with different sample types. Bold face: values indicating significant differences (after Bonferroni adjustment)

Sample	Mean	SD	Z	p
Control	3.111	2.247	-	-
AUC A	6.333	2.525	-2.771	**0.003**
CHC	6.222	1.978	-3.233	**0.001**
AUC B	6.203	1.996	-3.002	**0.001**
HAM Y	5.027	4.842	-0.808	0.209
HAM X	4.777	3.798	-0.577	0.281
TestoPeat	4.500	1.175	-1.616	0.053
HAM U	4.463	0.802	-1.732	0.041
AUC-H	4.333	0.854	-1.385	0.082
Peat	3.611	1.640	-0.346	0.364
HAM b	3.333	2.160	-0.230	0.408
HAM Z	2.952	2.791	0.205	0.418
HAM c	2.500	2.116	0.461	0.322

In order to test the similarities or differences between all tests the WRS probabilities for all combination of tests were calculated (Table 3.3). The resulting matrix is symmetrical about the diagonal. Several tests were significantly different from other tests ($p < 0.05$). The tests with dung from the males, AUC A, CHC, AUC B, and HAM U, form a group of test which are mostly compatible with each other but different from most other tests. Tests with peat sample and the samples from the juveniles, HAM b and HAM c, form another group together with the control

test. The tests with dung from the females HAM X, HAM Y, HAM Z, the heated sample AUC H and the peat sample with testosterone are in between the other two groups and have some overlap with both of these groups at the extremes.

Table 3.3. Spray urinating. Data from testing Cyrano (male rhino). Probabilities from Wilcoxon Sum Rank tests. Bold face: two samples significantly different from each other (P< 0.5)

	Contr	Peat	TesP	HAMb	HAMc	HAMY	HAMZ	HAMX	HAMU	CHC	AUCA	AUCB	AUCH
Contr	-	0.36	0.05	0.41	0.32	0.21	0.42	0.28	**0.04**	**0.00**	**0.00**	**0.00**	0.08
Peat	0.36	-	**0.03**	0.36	0.12	0.32	0.09	0.18	**0.03**	**0.00**	**0.00**	**0.00**	0.07
TestP	0.05	**0.03**	-	0.07	**0.01**	0.32	**0.03**	0.24	0.41	**0.01**	**0.01**	**0.00**	0.50
HAMb	0.41	0.36	0.07	-	0.18	0.41	0.27	0.24	0.10	**0.00**	**0.00**	**0.00**	0.12
HAMc	0.32	0.12	**0.01**	0.18	-	0.08	0.38	0.12	**0.01**	**0.00**	**0.00**	**0.00**	**0.03**
HAMY	0.21	0.32	0.32	0.41	0.08	-	0.21	0.41	0.32	0.12	0.12	0.12	0.24
HAMZ	0.42	0.09	**0.03**	0.27	0.38	0.21	-	0.09	**0.04**	**0.00**	**0.00**	**0.00**	**0.04**
HAMX	0.28	0.18	0.24	0.24	0.12	0.41	0.09	-	0.21	0.08	**0.03**	**0.04**	0.24
HAMU	**0.04**	**0.03**	0.41	0.10	**0.01**	0.32	**0.04**	0.21	-	**0.01**	**0.00**	**0.00**	0.32
CHC	**0.00**	**0.00**	**0.01**	**0.00**	**0.00**	0.12	**0.00**	0.08	**0.01**	-	0.41	0.50	**0.00**
AUCA	**0.00**	**0.00**	**0.01**	**0.00**	**0.00**	0.12	**0.00**	**0.03**	**0.00**	0.41	-	0.32	**0.02**
AUCB	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.12	**0.00**	**0.04**	**0.00**	0.50	0.32	-	**0.00**
AUCH	0.08	0.07	0.50	0.12	**0.03**	0.24	**0.04**	0.24	0.32	**0.00**	**0.02**	**0.00**	-

3.1.5. Other behaviour categories

In contrast to spray urinating, scanning, smelling the sample, smelling the ground, dragging, urinating, defecating, and overmarking (i.e., the other categories from the theme 'territorial') were executed by the females as well as males. In order to test all these cases for correlations between data for only for a single animal but from all tests, I applied the Kruskal-Wallis H Test (KWH). The results are probabilities which indicate whether the different data were drawn from the same distribution or whether they differ ($p < 0.05$). There were significantly differences in Cyrano's territorial performance of scanning, smelling sample, smelling ground, and dragging in the presence of different samples (Table 3.4). This was also true for the females with respect to scanning (Table 3.4). Smelling the ground behaviour of Utani and Smelling excrement of Cyrano occurred only 74 and 90 times during 79 observation sessions and, therefore, a meaningful statistical comparison is ruled out because of small sample size.

There were significant differences in the occurrence of locomotion among tests (Table 3.4). Even feeding by Cyrano was affected by the tests with different sample types. Analyzing the results of other behaviour categories (drinking, standing by Mapenzi, attacking and rubbing horns) are ruled out because of small sample sizes.

Table 3.4. Behaviour categories that were affected by different sample types. Total: for behaviour events during all tests. H: test statistic from Kruska-Wallis test

Animal	Category	Total #	H	p
Cyrano	Scanning	450	26.831	0.008
Cyrano	Smelling ground	644	50.629	0.000
Cyrano	Smelling sample	177	54.895	0.000
Cyrano	Smelling excrement	90	32.507	0.001
Cyrano	Dragging	175	48.126	0.000
Cyrano	Walking	1847	21.266	0.047
Cyrano	Feeding	1254	25.146	0.014
Cyrano	Drinking	34	28.747	0.004
Cyrano	Standing	171	37.796	0.000
Cyrano	Attack	71	40.227	0.000
Cyrano	Rub horn	55	22.022	0.037
Utani	Scanning	191	42.753	0.000
Utani	Smelling ground	74	22.542	0.032
Utani	Walking	379	24.014	0.020
Mapenzi	Scanning	293	28.224	0.005
Mapenzi	Walking	454	24.260	0.019
Mapenzi	Standing	101	22.204	0.035

Scanning and walking behaviour of all animals varied significantly during the tests with different sample types (Table 3.4). and, thus, were different than the other territorial behaviour categories. Therefore, I examined these behaviour categories and checked which samples caused the strongest effects (Tables 3.5). In the following I compare various types of behaviour of the females with the same behaviour of the male in response to the various sample types.

Summary of behavioural reactions to different faecal sample types

- **Spray urinating:** Very strong reaction by Cyrano to male samples. Weaker reaction to female and TestoPeat samples. No reaction to juvenile and control samples.

- **Scanning:** Strong reaction by Cyrano to male samples and to HAM Y. Weaker reaction by females to male samples and some reaction to juvenile and females samples

- **Smelling the sample:** Strong reaction by Cyrano and Utani to the male samples CHC, AUC A, and AUC B. Strong reaction by Mapenzi to Peat and TestoPeat. Strong reaction by Utani and Cyrano to HAM Y.

- **Smelling the ground:** Strong reaction by all animals to male samples. Some reaction to TestoPeat by Mapenzi and Cyrano
- **Dragging:** Some reaction by Cyrano to all male samples
- **Walking:** Strong reaction by Cyrano to male samples. Some reactions by females to male samples.
- **Feeding:** Reduced feeding time by Cyrano in reaction to some male samples, TestoPeat and heated sample. No conspicuous reaction by females to male samples.

In summary, there was strong reaction by Cyrano to male samples. Reactions by females to male samples were significantly different from random, but seemingly weaker than Cyrano's reactions to male samples. In some cases, there were also reactions to some female and juvenile samples, and there are a few cases of reactions to TestoPeat and peat samples.

Table 3.5. Number of occurrence (No) and total time (sec) per session for various behaviour categories for Mapenzi (MP), Utani (UT), and Cyrano (CY). Where there were fewer than 9 trials, both values corrected for 9 trials. Longest times marked by bold numbers. Tabulated separately for Scanning and for Walking

a) Scanning

	MP		UT		CY	
	No.	**time**	**No.**	**time**	**No.**	**time**
Control	102.	292.5	84.	**251.0**	105.	191.5
Peat	57.	424.0	21.	125.0	31.	97.3
TestoPeat	57.	514.5	27.	106.0	39.	129.0
HAM b	102.	**896.0**	89.	**342.7**	32.	111.2
HAM c	39.	199.0	24.	75.0	27.	130.5
HAM Y	129.	**580.5**	81.	279.0	81.	**229.5**
HAM Z	42.	528.4	84.	**316.7**	51.	153.8
HAM X	78.	281.0	21.	47.5	66.	166.0
HAM U	39.	326.0	30.	217.0	36.	174.5
CHC	102.	**561.0**	36.	124.5	71.	**206.3**
AUC A	45.	277.0	9.	40.0	64.	177.6
AUC B	72.	507.0	24.	95.0	49.	205.3
AUC-H	33.	310.0	45.	184.5	40.	**215.5**

Table 3.5. continued

b) Walking

	MP		UT		CY	
	No.	time	No.	time	No.	time
Control	138.	**626.0**	93.	392.0	186.	833.5
Peat	96.	471.0	57.	421.5	160.	863.7
TestoPeat	93.	460.5	66.	468.5	191.	1179.0
HAM b	110.	486.5	125.	**542.5**	128.	777.0
HAM c	90.	526.0	51.	379.5	135.	1058.5
HAM Y	111.	477.5	108.	**542.5**	206.	946.5
HAM Z	90.	241.9	81.	290.1	174.	909.4
HAM X	99.	313.0	87.	358.0	210.	592.0
HAM U	120.	**654.0**	120.	**729.0**	195.	937.0
CHC	132.	578.0	78.	306.0	261.	**1245.2**
AUC A	84.	523.5	72.	335.5	219.	960.7
AUC B	126.	**633.0**	102.	462.5	262.	**1341.8**
AUC-H	99.	**772.5**	102.	**636.0**	215.	**1445.8**

3.1.6. Synchronous behaviour

Considering data obtained by the scan sampling method, there were ten times during an observation session when I recorded the behaviour of all animals, with two examples being shown in Fig. 3.14. Several times during the session, two animals had the same behaviour (Fig. 3.15). Although less common, there were also instances of all rhinos adopt the same behaviour. By far the most frequent synchronous behaviour was feeding (75%), followed by walking (10%) and lying down (10%).

The number of synchronous behaviour states varied between tests (see Table 3.6 for mean and SD). Numbers for the pairs containing Cyrano and one of the females varied from 5.9 to 7.5 out of the 10 observed behaviour states. The numbers of synchronous states of the pair Utani and Mapenzi was significantly higher, varying from 7.3 to 9.0. The numbers of synchronous states of all animals varied from 4.8 to 6.8.

For the pair Cyrano – Utani, tests with sample types HAM Z were significantly different (WRS tests) from most other tests, while tests with Control, Peat, HAM c, HAM Y, HAM U, and AUC H were compatible to all other tests. For the pair Cyrano – Mapenzi, tests with sample types Control, Peat, HAM c, HAM Y, HAM U, and AUC H were comparable to all tests. For the pair

Utani – Mapenzi, tests with sample types HAM c, and HAM Z had distributions different from most other tests, while tests with Control, HAM Y, and HAM U were comparable to all tests. For three animals, tests with sample types AUC B were different from most other tests, while tests with Peat, TestoPeat, HAM c, HAM Y, HAM U, and AUC H were comparable to all tests. The tests with samples not mentioned above were comparable to some tests and not to others. On the whole, this analysis showed that there was variation in the numbers of synchronous states displayed, suggesting effects of the different samples. But a clear picture which group of samples had the strongest effect has not emerged. Therefore, in the next section, I consider another aspect of synchronous behaviour, the distance between animals.

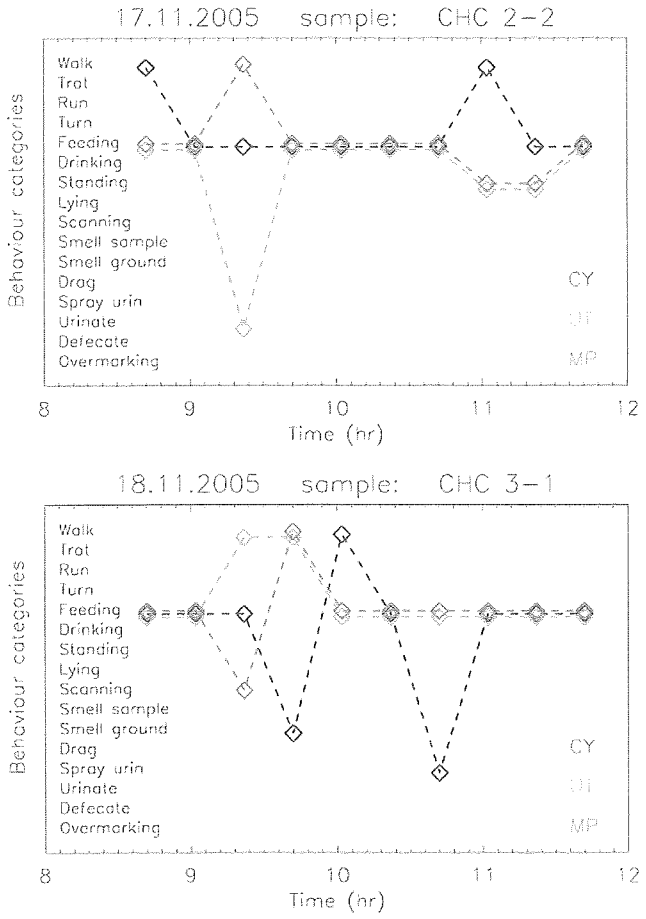

Figure 3.14. Examples of synchronous behaviour on 17 and 18 November 2005 when samples from the Christchurch bull were used. Diamonds mark the behaviour category observed at the time given (for better visibility diamonds are shifted by a small distance). Different animals are colour coded, dashed lines connect behaviour states of individual subjects.

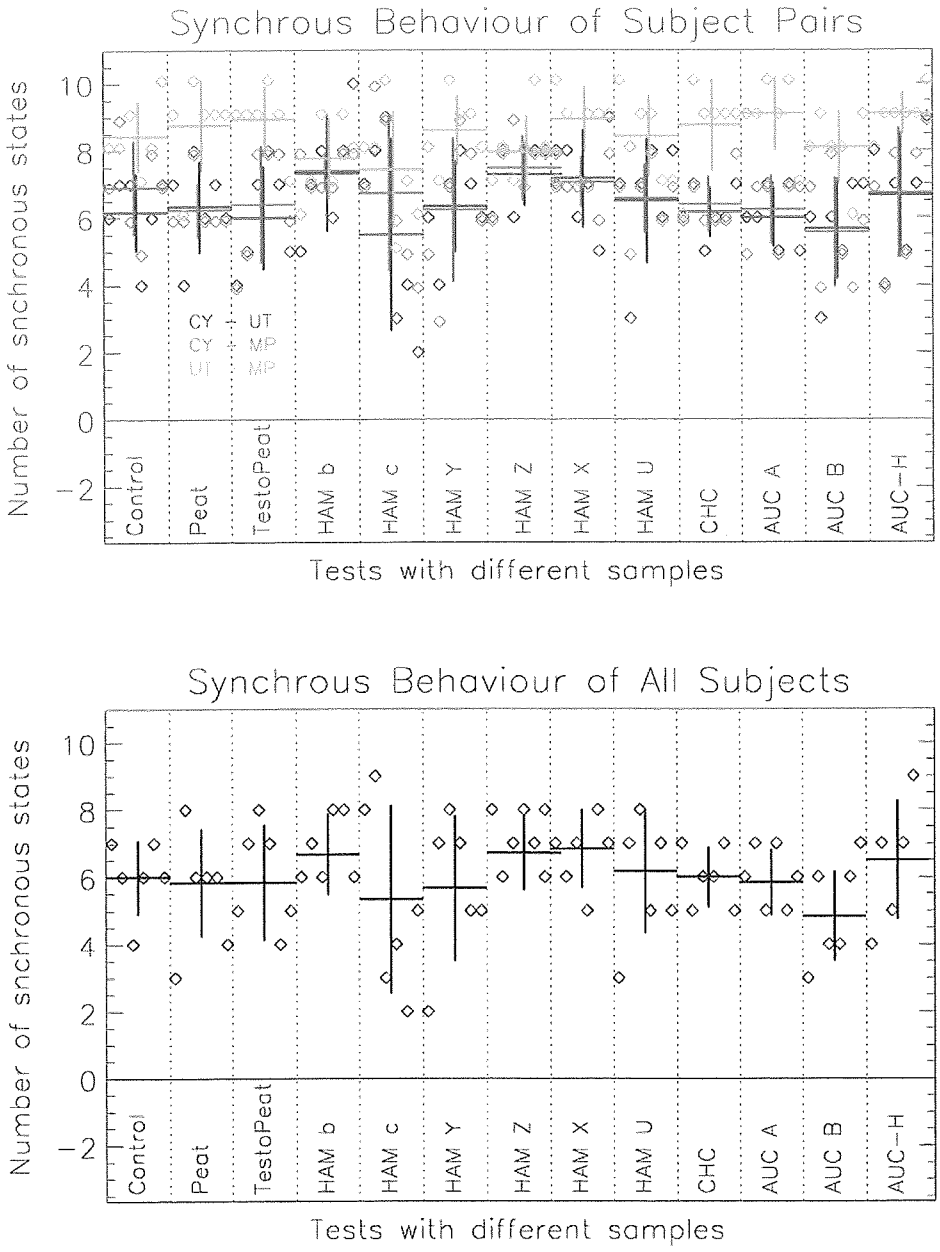

Figure 3.15. Synchronous behaviour statistics. Diamonds mark values of individual sessions. Thick crosses indicate the mean frequencies for specific tests (i.e. sample types) and standard deviations. Upper panel: Synchronous behaviour of pairs of animals (colour coded). Lower panel: Synchronous behaviour of all three animals.

Table 3.6. Numbers of synchronous behaviour states of pairs of animals and of all animals together. CY-UT: pair Cyrano (CY) and Utani (UT). CY-MP: Cyrano (CY) and Mapenzi (MP). Etc.

	CY-UT		CY-MP		UT-MP		All animals	
	mean	SD	mean	SD	mean	SD	mean	SD
Control	6.17	1.17	7.00	1.41	8.33	1.03	6.00	1.10
Peat	6.33	1.37	6.33	0.82	8.67	1.37	5.83	1.60
TestoPeat	6.00	1.55	6.50	1.76	8.83	0.98	5.83	1.72
HAM b	7.33	1.75	7.50	0.55	7.67	1.21	6.67	1.21
HAM c	5.50	2.88	6.83	2.32	7.33	1.75	5.33	2.80
HAM Y	6.33	1.37	6.33	2.16	8.50	1.05	5.67	2.16
HAM Z	7.29	0.95	7.57	0.98	7.86	1.07	6.71	1.11
HAM X	7.17	1.47	7.17	0.75	8.83	0.98	6.83	1.17
HAM U	6.50	1.87	6.67	1.03	8.33	1.21	6.17	1.83
CHC	6.17	0.75	6.50	0.84	8.67	1.37	6.00	0.89
AUC A	6.00	0.89	6.33	1.03	9.00	1.10	5.83	0.98
AUC B	5.67	1.51	5.67	1.63	8.00	1.10	4.83	1.33
AUC-H	6.67	1.86	6.83	1.94	9.00	0.63	6.50	1.76

3.1.7. Distances between animals

Distances between animals were measured ten times per observation session. The mean distances per session varied between one and 15 body lengths (ca. 4 m, Fig. 3.16, Table 3.7). The SD of the distance is about 25% of the mean except for the test with the TestoPeat samples where it reached 50%. Because of this large error, I used the median distance for representing distances between the animals in the various tests. The distance between Cyrano and the two females was 6.3 body lengths and the distance between the two females was 2.5 body lengths.

Using WRS tests for comparing the various data sets with the data from control tests, the distances between Cyrano and the females in almost all tests were significantly different. The exception was when comparing Peat and HAM Y samples. However, there was no significant difference in the distances between the females in tests with the different sample types.

Table 3.7. Mean (plus SD) distances in body length (ca. 4 m) between rhinos. Cyrano (CY). Mapenzi (MP). Utani (UT).

	CY-UT		CY-MP		UT-MP	
Sample	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
Control	3.50	0.80	4.05	0.77	1.7667	0.60
Peat	3.45	1.83	5.10	1.97	2.3167	1.60
TestoPeat	8.40	4.67	7.56	3.97	2.41	1.53
HAM b	7.40	1.88	7.03	2.21	3.81	2.50
HAM c	6.58	1.24	7.25	1.8577	3.68	1.92
HAM Y	3.66	0.81	4.25	0.8456	1.65	0.58
HAM Z	5.04	1.39	5.70	1.3329	3.21	1.44
HAM X	4.73	1.22	5.93	0.8524	2.35	1.23
HAM U	4.96	1.61	6.01	1.9854	2.48	1.17
CHC	6.56	2.61	6.85	2.8247	3.15	1.40
AUC A	6.21	2.87	6.43	2.6927	1.76	0.73
AUC B	10.23	3.82	10.50	3.4802	2.75	1.81
AUC-H	8.8	2.29	8.85	1.5003	3.08	1.07

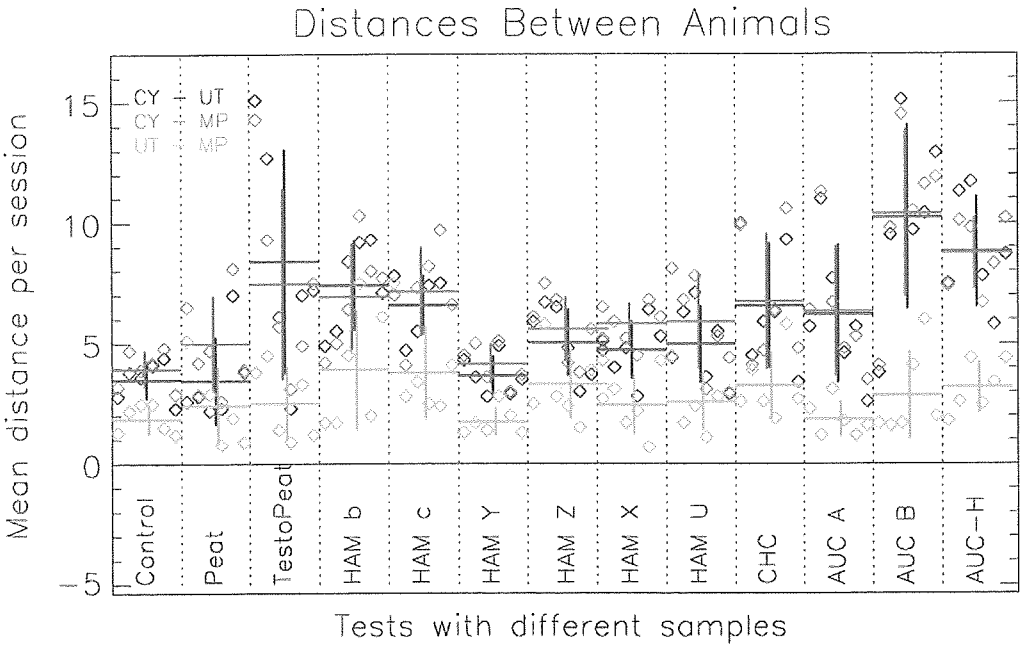

Figure 3.16. Mean distance in body length (ca. 4 m) between animals per observation session (diamonds). Thick crosses give mean values and standard deviation per sample type.

Four groups were evident when distances between Cyrano and the females are considered across the different tests. The groups of tests in which there were significantly different distances ($p < 0.05$) between Cyrano and the females are listed below.

1. Control, Peat, HAM Y
2. HAM Z, HAM X, HAM U
3. HAM b, HAM c, CHC, AUC A
4. TestoPeat, AUC B, AUC H

Groups 1 and 4 were significantly different from each other, but groups 2 and 3 overlapped considerably.

3.1.8. Synchronous behaviour at close distance

For detecting synchronous behaviour at close distances, I used a subset of the data (i.e., I made a requirement that the distance between animals had to be closer than the median value of the distance between two animals) (Fig. 3.17, Table 3.8). The lowest numbers of synchronous states between Cyrano and the females were found in the tests with the samples from the males CHC, AUC A, AUC B, and AUC H, and also with samples from the juvenile HAM c (Fig. 3.17). These tests also form a group with distributions similar to each other, but different from the distributions from testing with other samples. Apparently synchronous behaviour is affected by samples from males.

3.2. Chemical Analysis of Dung Samples

3.2.1. Olfactory components

In an initial gas chromatography survey of the samples, retention time spectra were obtained for selected molecular mass lines. Using GC-MS, 14 different volatile compounds were identified (Fig. 3.18). There was a major influx in the ion count for masses first appearing at 2:32 min after sample injection (time 0). Fourteen peaks were identified up to retention time 35:20 min, corresponding to different volatile compounds (Table 3.9).

Table 3.8. Number of synchronous behaviour states when rhinos were closer than their median distances. Cyrano (CY). Mapenzi (MP). Utani (UT).

	CY - UT		CY - MP		UT - MP	
	mean	**SD**	**mean**	**SD**	**mean**	**SD**
Control	5,50	1,64	5,66	1,21	6,66	1,36
Peat	5,83	1,72	5,00	1,26	7,66	1,50
TestoPeat	4,50	2,07	5,33	1,96	6,16	2,31
HAM b	4,66	1,21	5,00	2,09	5,33	1,75
HAM c	3,66	2,42	3,83	2,48	4,50	1,64
HAM Y	5,66	1,03	5,16	1,72	7,33	0,81
HAM Z	5,28	1,38	5,42	1,27	4,57	1,71
HAM X	5,66	1,50	4,66	1,03	6,50	1,76
HAM U	5,66	2,16	4,83	1,94	6,83	2,13
CHC	4,50	1,64	4,66	1,86	5,50	2,07
AUC A	3,83	1,60	4,33	1,03	7,16	1,32
AUC B	3,50	1,04	3,83	1,72	6,50	1,76
AUC-H	4,16	0,75	4,16	0,98	6,50	1,51

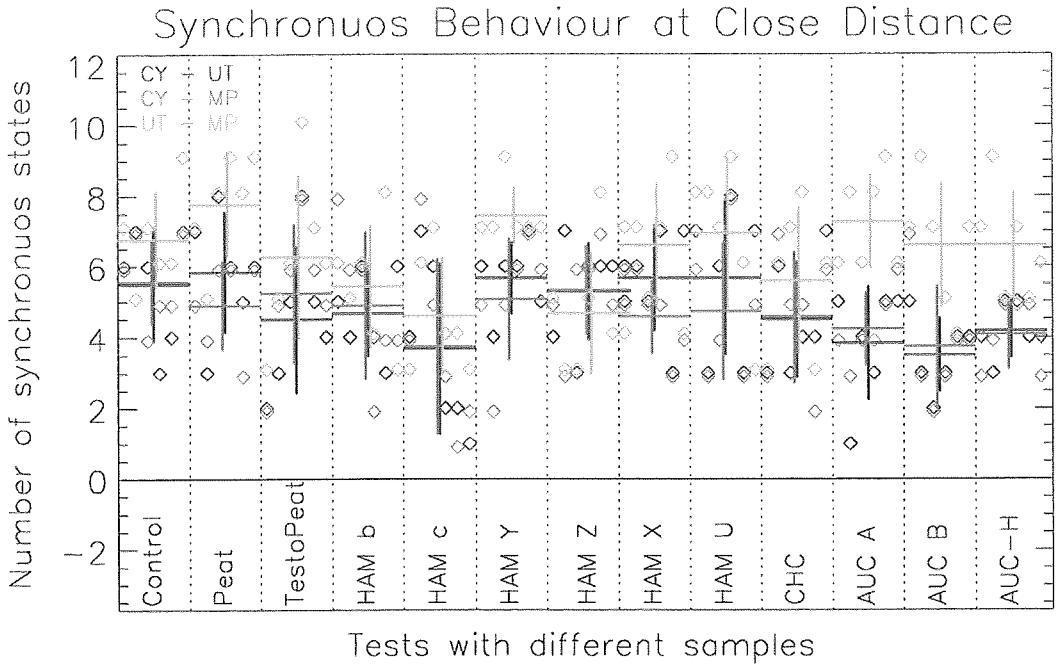

Figure 3.17. Synchronous behaviour of pairs of animals at distances closer than the median distances (diamonds). Thick crosses give mean values and standard deviation per sample type.

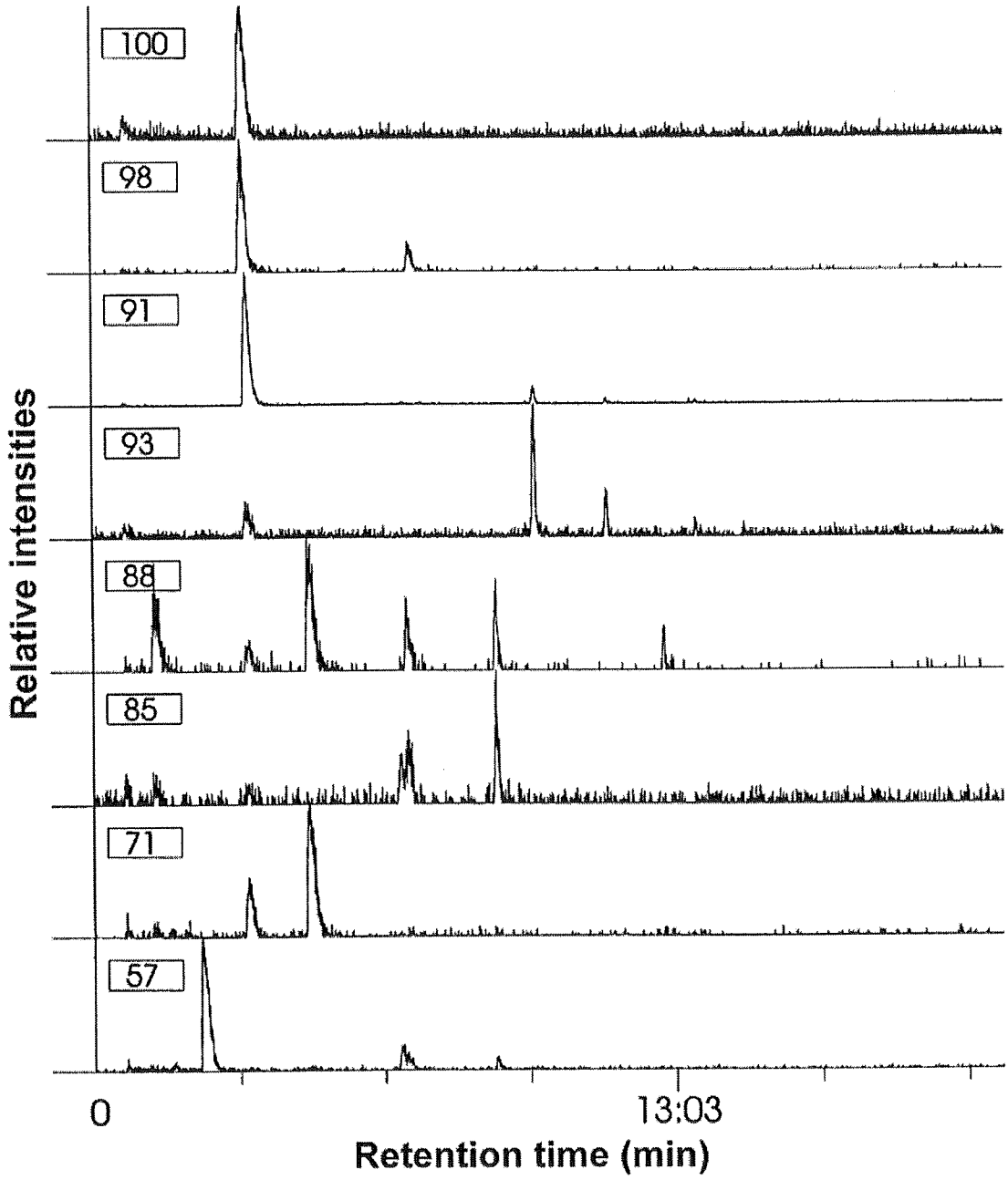

Figure 3.18. GC-MS raw data of sample HM 12 A. Evolution of key mass lines (at 57, 71, 85, 88, 91, 93, 98 and 100 dalton) as function of retention time. Gas injection occurs at time 0. Peak heights are normalized to the maximum height.

Table 3.9. Retention times and associated molecular mass lines from GC-MS of rhino faecal samples. 1: Survey measurements. 2: detailed analyses

Time min	2:32	3:37	5:03	6:30	9:15	9:41	10:04	11:40	13:10	18:30	19:00	24:00	26:15	26:35	28:30	30:00	35:20
Mass dalton																	
57	2																
60		1		1					1	1		1					
71			2														
85					2												
88					2												
91							1			1				1		1	
93						1	2	2					1		1	1	1
98				1	1						1						
100					1												
136							2	2					1			1	1

The gas chromatograms of different samples varied considerably, especially for samples from different donor animals. Therefore, means were derived for the total ion contents (peak area) from all measurements of samples from a specific donor animal (Table 3.10). Means over different sexes have also been determined for the major important retention times (Table 3.11). As the coefficients of variation were about 30%, I judged differences between means to be substantial only when the means are about a factor of two apart.

As the strongest reactions by rhinos were to dung from males, components from these samples were examined in more detail. At 9:15, 9:41, and 13:10 min, mean retention times for the bulls from Christchurch and Auckland were, by a factor two, higher than the values of the other rhinos. The Hamilton bull does not follow this trend. Females, and also juveniles, had higher peaks than males at retention times 18:30 and 19:00 minutes, while juveniles also had significant peaks there.

Table 3.10. Mean values of ion content (normalized to largest value at given retention time) in retention time peaks of samples from all rhinos. Bold: dominant peaks

	Retention times (min:sec)							
Min:Sec	**2:32**	**3:37**	**5:03**	**6:30**	**9:15**	**9:41**	**10:04**	**11:40**
CM 1	**0.281**	0.032	**0.198**	0.007	**0.234**	**0.510**	**0.410**	**0.306**
AM 1	**0.375**	0.025	**0.273**	0.007	**0.205**	**0.915**	0.120	0.064
AM 2	**0.577**	0.024	**0.543**	**0.103**	**0.544**	**0.514**	0.000	0.000
HM 1	0.014	0.024	0.006	0.011	0.000	0.017	0.000	0.000
HF 1	0.018	0.017	0.009	0.012	0.000	0.017	0.000	0.000
HF 2	0.025	0.024	0.011	0.012	0.000	0.017	0.000	0.000
HF 3	0.008	0.026	0.000	0.022	0.000	0.013	0.208	0.000
HJ 2	0.021	0.029	0.010	0.024	0.010	0.023	0.000	0.000
HJ 3	0.018	0.016	0.005	0.015	0.000	0.022	0.129	0.000

Table 3.10. continued

	Retention times (min:sec)								
Min:Sec	**13:10**	**18:30**	**19:00**	**24:00**	**26:15**	**26:35**	**28:30**	**30:00**	**35:20**
CM 1	**0.053**	0.022	0.052	0.047	**0.157**	0.082	0.015	0.115	0.068
AM 1	**0.114**	0.027	0.012	0.071	0.042	0.024	0.005	0.020	0.030
AM 2	**0.077**	0.021	0.008	0.071	0.035	0.017	**0.032**	0.017	0.015
HM 1	0.014	0.036	0.039	0.009	0.072	0.039	0.008	0.065	0.053
HF 1	0.010	0.032	0.028	0.021	0.058	0.046	0.010	0.087	0.048
HF 2	0.007	0.036	0.025	0.008	0.023	0.023	0.015	0.032	0.042
HF 3	0.020	0.042	**0.063**	0.007	**0.123**	0.067	0.011	0.033	0.055
HJ 2	0.028	**0.062**	0.029	**0.720**	0.057	0.044	0.014	0.043	0.050
HJ 3	0.008	**0.058**	**0.060**	0.005	**0.140**	0.071	0.012	0.079	0.060

Table 3.11. Mean values of ion content in retention time peaks of samples from animals of different sexes.

	Retention times (min:sec)			
Min:Sec	**2:32**	**5:03**	**9:15**	**10:04**
Male	0.291±0.130	0.231±0.111	0.218±0.109	0.141+0.110
Female	0.015±0.004	0.006±0.003	0.000±0.000	0.080+0.111
Juvenile	0.020±0.005	0.008±0.003	0.006±0.005	0.070+0.093

Using GC-MS and choosing a subset of samples from males, females and juveniles, I identified the compounds of these retention time peaks. Three interesting peaks emerged, at 2:32, 3:37, and 5:03 min. By using Wiley's Database for determining relative similarity (Table 3.12), candidate components of faecal volatiles at given retention times were identified from the mass spectra (Fig. 3.19). Chemicals were listed only if they had a relative similarity greater than 60%, as computed by the GC-MS analytical software. Retention time peaks at 9:41 and later than 13:10 min could not be identified, because either no match was found with a similarity larger than 60%, because neighbouring peaks could not be separated, or because there were overlapping mass spectra. The retention time peak at 13:10 min was a consequence of contamination from the silicone rubber plugs during sample preparation. Identified chemicals included esters of carboxylic (fatty) acids, terpenoids, and toluene.

Among the identified chemicals were three esters of carboxylic acids. The typically high volatility of these compounds suggests that they might function for the rhinos as signals. They all consist of short chain acids (3. 4. and 5 carbon atoms) to which an ethyl ester is attached, and

they all have distinct fruity/floral odours. There were also some terpenoids, with these being compounds that are characteristically plant by-products, often being found in the food of grazing herbivores. Finally some unusual contaminants were unlikely to be biologically relevant to this study (e.g., silicone rubber is used to plug the sample vials, and toluene is a common industrial and vehicle pollutant in city air).

Table 3.12. Identified volatiles in rhino faecal samples. Retention time (min: sec), Molecular weight: Dalton. Rel. sim.: relative similarity in %.

Retention Time	Molecular Weight	Chemical Formula	Common Name	Chemical	Rel. Sim.
2:32	102	$C_5 H_{10} O_2$		Propanoic acid, ethyl ester	93
3:37	92	$C_7 H_8$	Toluene	Benzene, methyl-	98
5:03	116	$C_6 H_{12} O_2$		Butanoic acid, ethyl ester	99
9:15	130	$C_7 H_{14} O_2$		Pentanoic acid, ethyl ester	93
10:04	136	$C_{10} H_{16}$	Terpenoids	(-)-trans-2-Carene	96
				1-Limonene	94
				(-)-cis-2-Carene	94
				Trans-Ocimene	94
				4-Carene	93
				1,3,6-Octatriene, 3,7-dimethyl-, (E)-	93
				Cis-Ocimene	93
				.alpha.-Pinene, (-)-	93
				.delta.3-Carene	93
				Cyclohexene, 1-methyl-5-(1-methlethenyl)-	91
				Tricyclo 2.2.1.02,6!Heptane, 2,3,3-Trimethyl-	90
				.beta.-Terpinene	90
				.alpha.-Thujene	90
				Bicyclo 3.1.1!hept-2-ene, 3,6,6-trimethyl-	70
				.beta.-Phellandrene	68
				1-Phellandrene	60
13:10	296	$C_8 H_{24} O_4 Si_4$	Silicone rubber	Cyclotetrasiloxane, octamethyl-	93
13:10	281	$C_{16} H_{12} Cl N_3$		1-Amino-1-ortho-chlorophenyl-2-(2-quino)	92

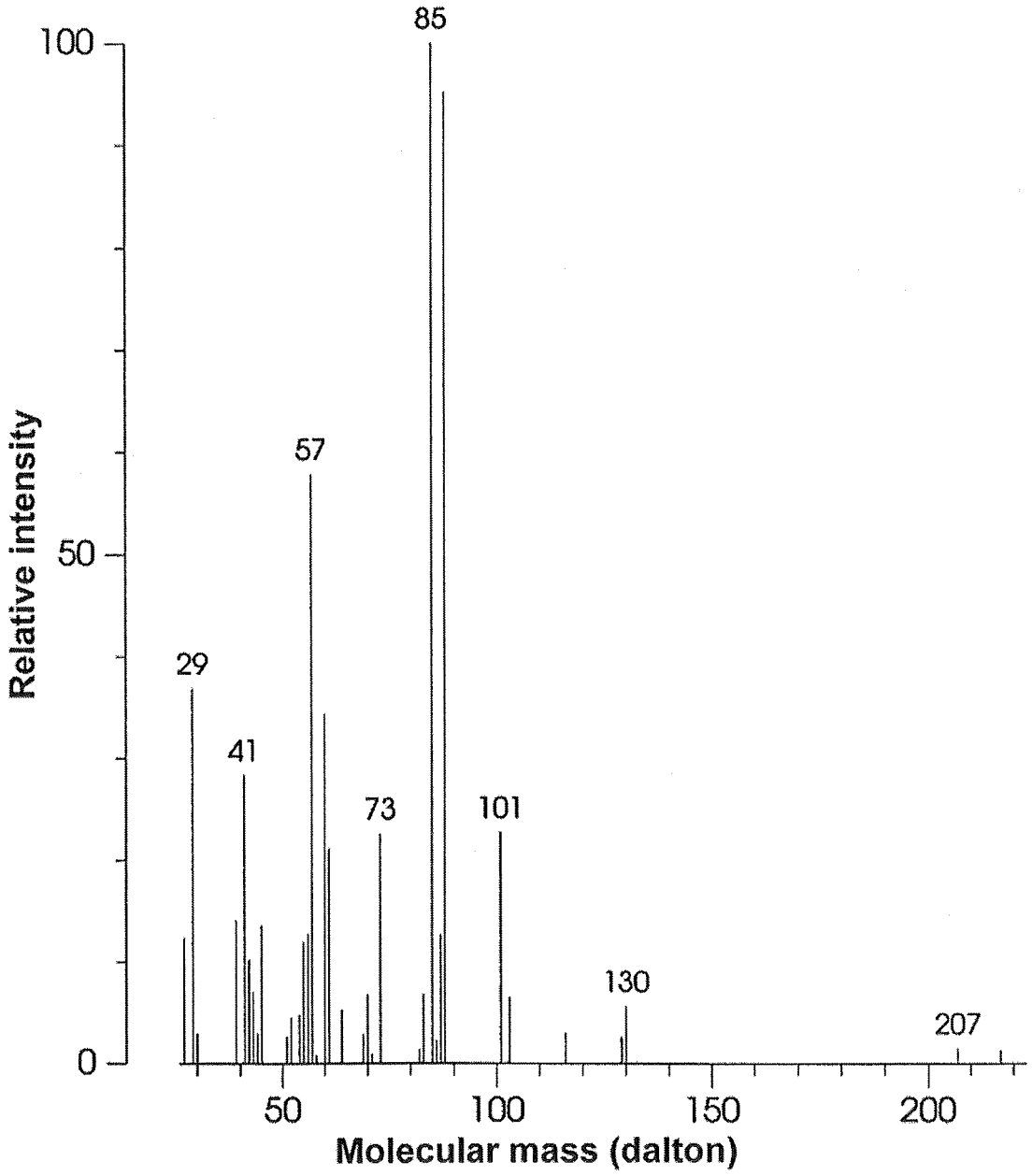

Figure 3.19. Mass spectrum at 9:15 min retention time. Major peaks are indicated by their molecular weight (dalton). The peak heights are normalized to the maximum height. Spectrum probably represents the fragmentation pattern of pentanoic acid, ethyl ester.

3.2.2. Testosterone analysis

Testosterone concentrations were determined for a) 43 dung samples from four rhino bulls, b) for peat samples that were impregnated with testosterone and c) for various control samples (Fig. 3.20). Runs 1 and 2 refer to the analysis of concentrations of testosterone including its metabolites. Run 1 was repeated in order to check the rather large variations between the individual samples. Run 3 was an analysis of testosterone concentration alone.

The testosterone levels of the samples varied by a factor more than three, but measurements from the same sample type were represented by their medians (Table 3.13). While the measurements of Run 1 and 3 were verified by control measurements, Run 2 had no controls, so the results for Run 3 were less certain than the others. For further analyses, I used the absolute concentrations of testosterone and its metabolites from Runs 1 and 3. Samples from the Hamilton bull had the highest testosterone and metabolites concentration (47.9 ng/g) which was close to that of the TestoPeat sample. Samples from the other bulls had values around 30 ng/g.

Although the deviations of the individual values are rather large, variation in the concentrations of testosterone (and its metabolites) are correlated between the different Runs (Fig. 3.21), and hence refer to true variations in the testosterone concentrations. Also the testosterone concentrations in dung from Cyrano varied somewhat during the course of the tests.

Tab. 3.13. Testosterone analyses of faecal samples types and controls. No.: number of samples used. Median: testosterone level (ng/g)

	Run 1		**Run 2**		**Run 3**	
Sample	**No**	**median**	**No**	**median**	**No**	**median**
Ctrl 1	5	4.9	0	./.	3	12.7
Ctrl 2	5	3.5	0	./.	3	14.4
Ctrl 3	5	5.6	0	./.	3	17.5
HAM U	6	47.9	6	58.0	6	13.0
CHC	7	24.5	8	32.9	8	9.3
AUC A	6	29.0	6	60.3	6	8.3
AUC B	7	36.7	9	50.2	9	7.1
TestoPeat	6	46.8	6	63.5	6	12.3
AUC DRY	3	46.3	5	38.0	5	6.0
Ctrl. HAM	2	23.6	2	45.5	2	11.7
Cyrano	12	14.9	12	46.8	12	13.5

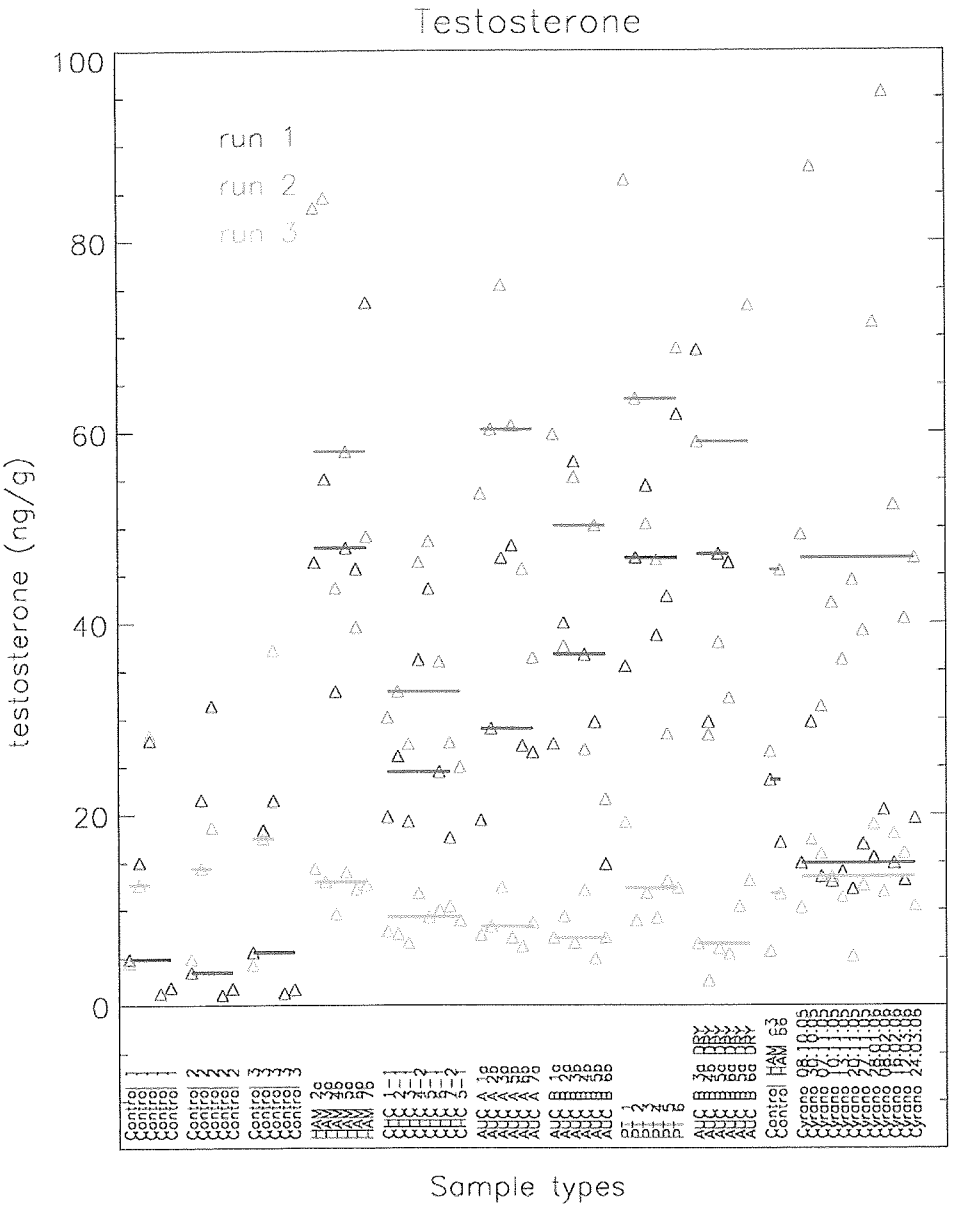

Figure 3.20. Testosterone concentrations of various faecal samples. Both values for individual samples (diamonds) and median values for each sample type (thick bars) are shown. Runs 1 an 2 refer to concentrations of testosterone and its metabolites, Run 3 refers to testosterone alone.

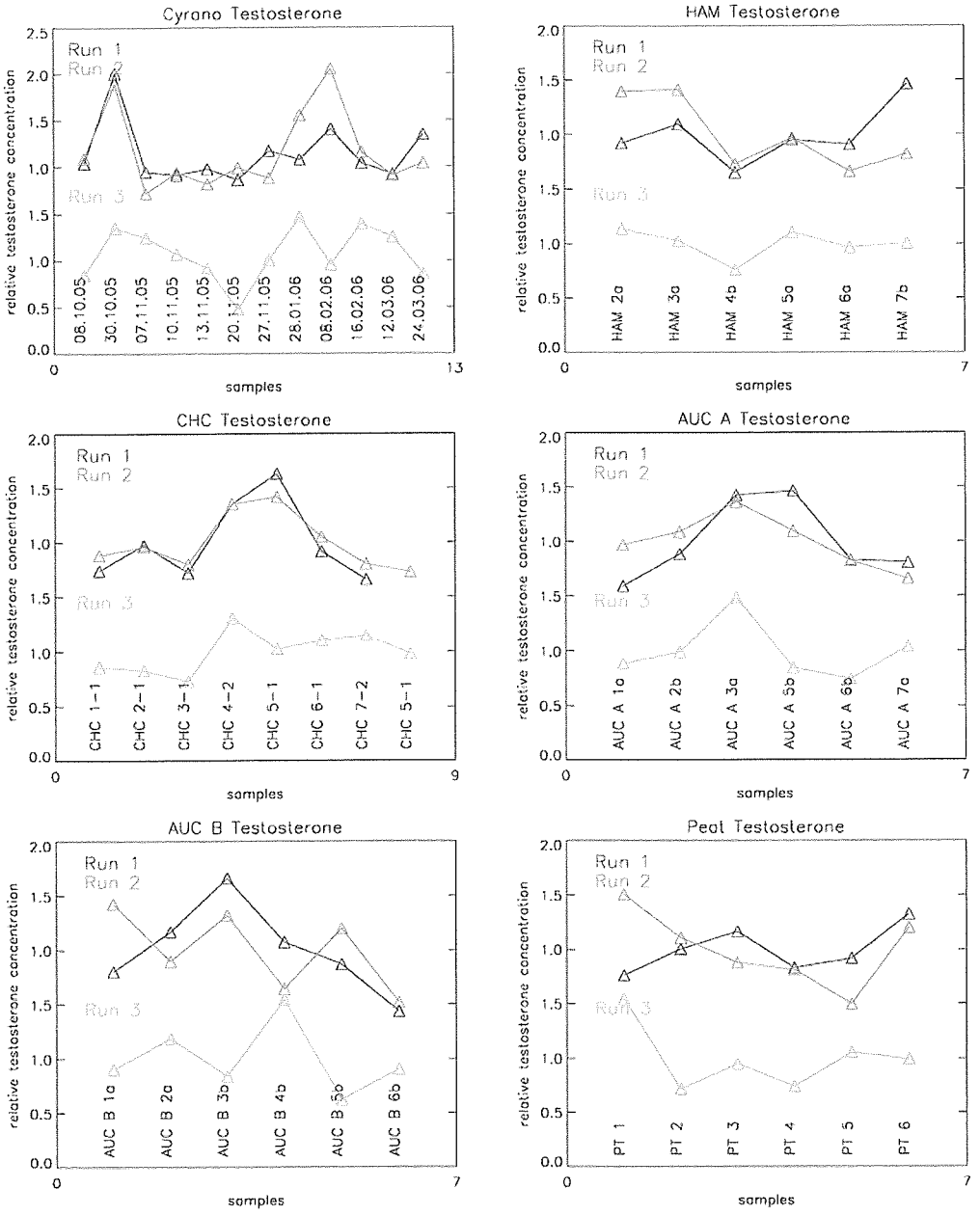

Figure 3.21. Individual testosterone concentrations normalized to the median of the testosterone concentration in dung from animals Cyrano, HAM, CHC, AUC A, AUC B, and PT. Runs 1 an 2 refer to concentrations of testosterone and its metabolites, while Run 3 refers to testosterone alone. Runs 1 and 2 are on the same ordinate while Run 3 is offset.

3.2.3. Effects of various chemical compounds

For determining which constituents in the sample potentially affected the behaviour of the test animals. I looked for a correlation between behavioural responses and the concentration of specific compounds. The strongest behavioural response to the samples was spray urinating by Cyrano. Therefore, I examined the dependency of the frequency of this behaviour with the concentrations of all chemical constituents identified and measured in the sample. The simplest dependency between the variables x and y is a linear dependency of the form

$$y = a + b \cdot x \qquad (2)$$

where a and b are constants.

Spray urinating varied with concentrations of testosterone (and metabolites, Run1 (Fig. 3.22). Although the fit is formally within the uncertainties of the measurements, it is unsatisfactory because the slope (b = 0.70) is very flat and its probable uncertainty (σ_b = 1.65) is larger than the slope itself (Table 3.14). Only the ethyl esters (Figure 3.23) had sufficiently small uncertainties to accept the linear dependency model. The terpenoids again gave no acceptable fit (Fig 3.24).

Table 3.14., Fit parameters and their probable uncertainties σ for various compounds found in the dung samples

Compound	a	σ_a	b	σ_b
Testosterone + metabolites	4.20	1.40	0.38	1.58
Testosterone	4.36	1.14	0.22	1.48
Propanoic acid ethyl ester	4.12	0.56	2.65	1.80
Butanoic acid, ethyl ester	4.18	0.54	2.67	1.91
Pentanoic acid, ethyl ester	4.20	0.54	2.65	1.91
Terpenoid	4.44	0.66	0.95	1.97

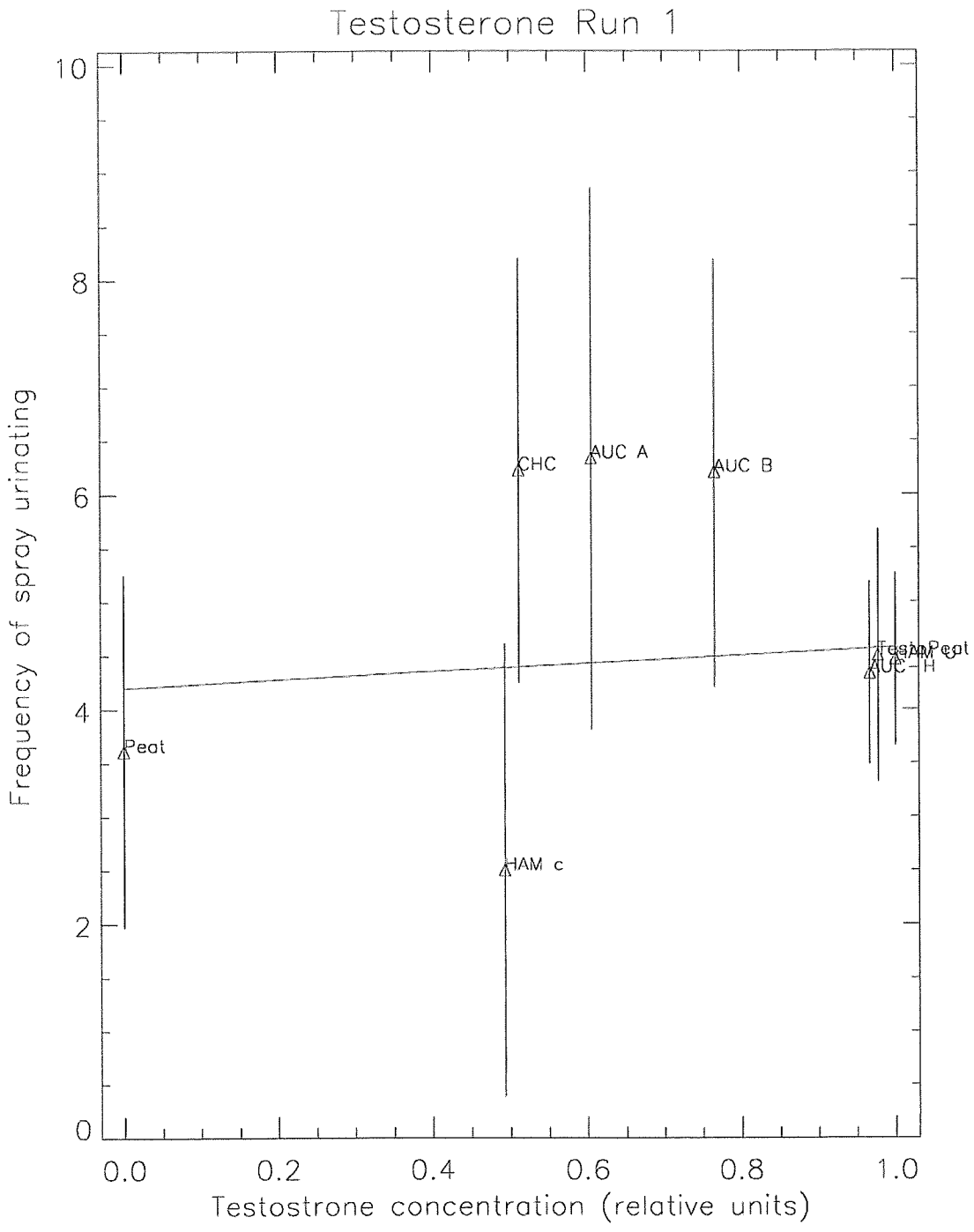

Figure 3.22. Mean frequency of spray urinating events per trial as function of testosterone concentration (Run 1). Linear fit (red) to data points.

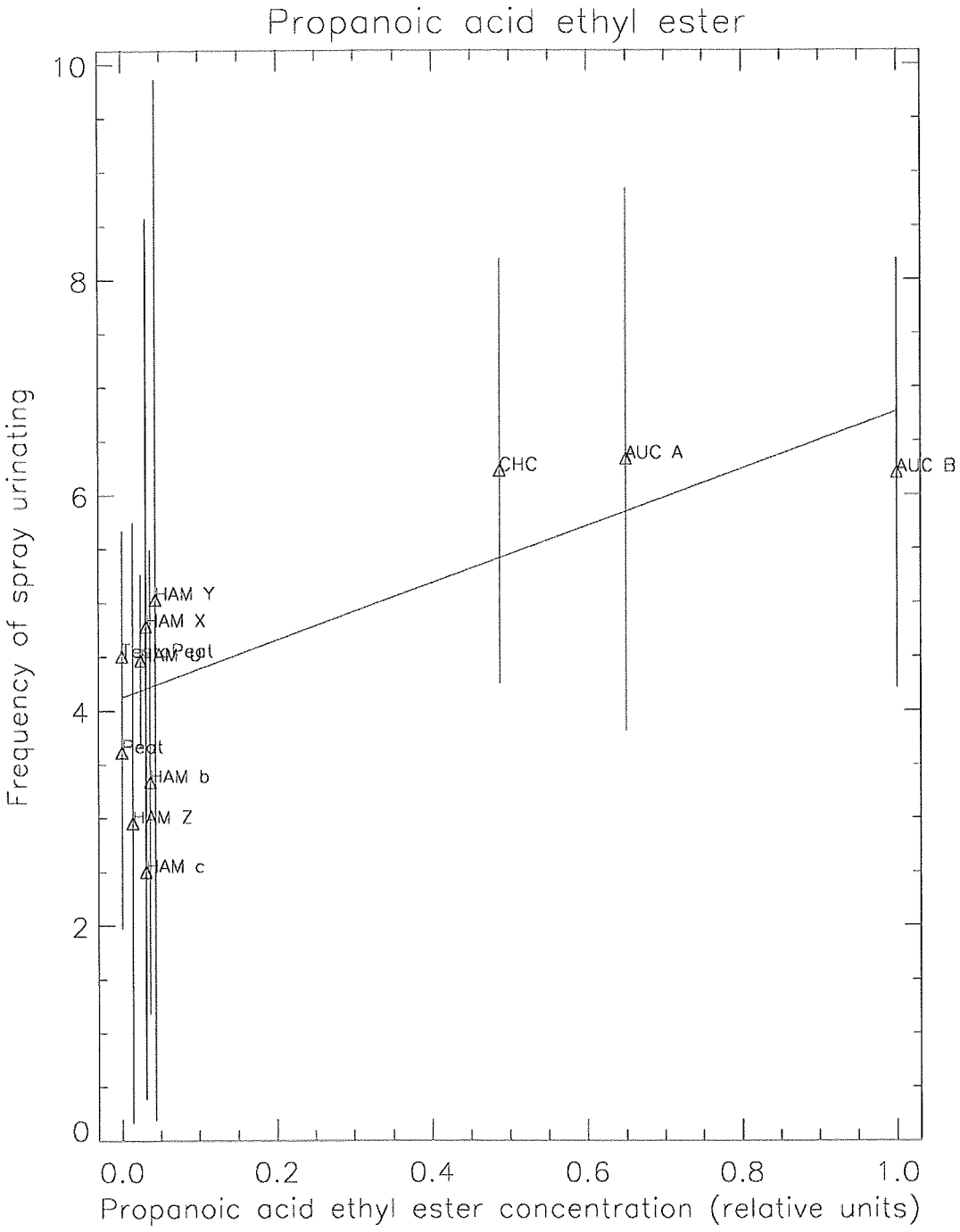

Figure 3.23. Mean frequency of spray urinating events per trial as function of the Propanoic acid, ethyl ester concentration. Linear fit (red) to data points.

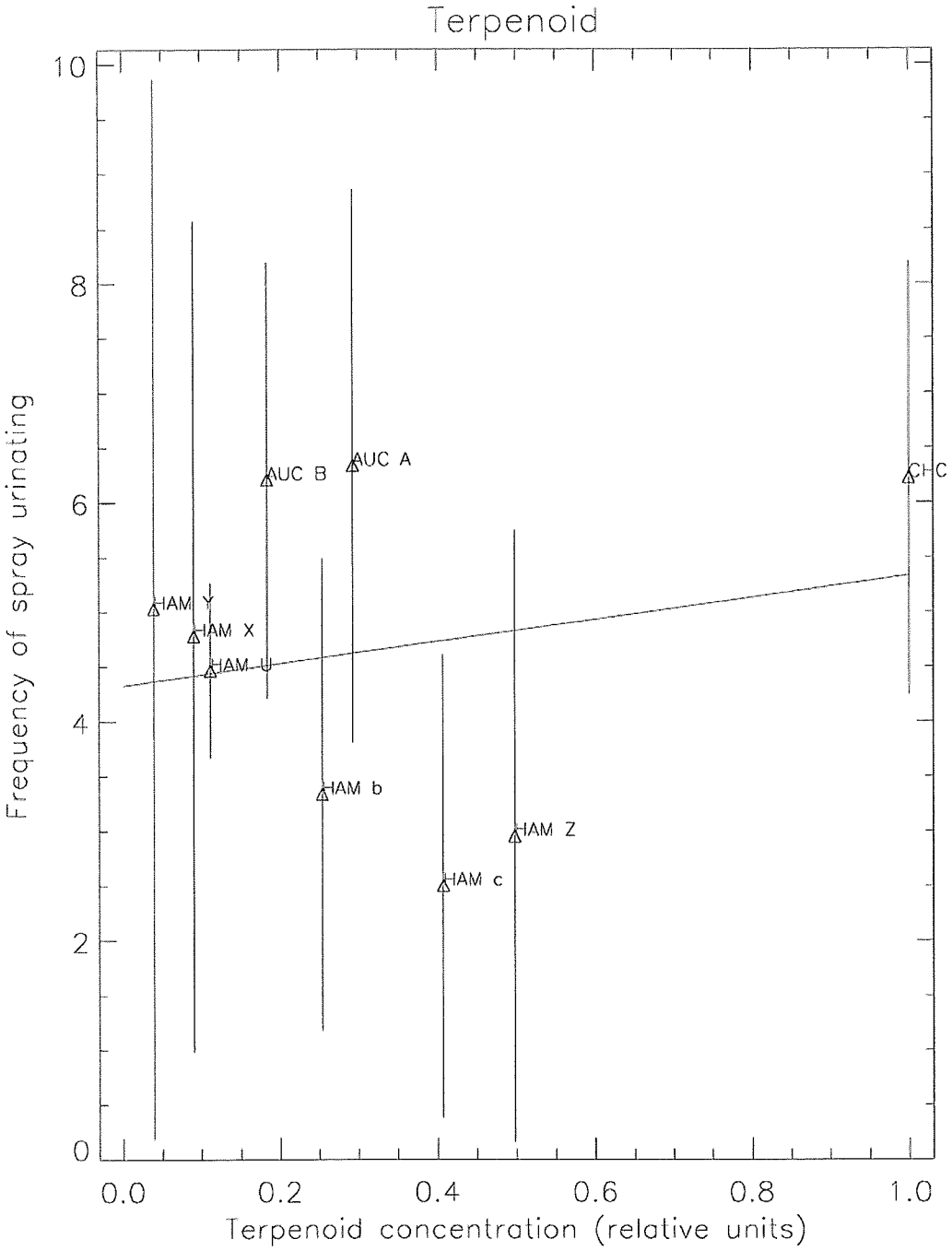

Figure 3.24. Mean frequency of spray urinating events per trial as function of Terpenoid concentration. Linear fit (red) to data points.

3.3. Environmental Conditions

Weather data for all observation sessions from the end of September 2005 to the end of March 2006 varied considerably (Fig. 3.25). For example, the noon temperatures varied between 9 and 29°C. From 9 A.M. to noon, the temperature typically increased by 5 to 10°C. However, there were also days when the temperature stayed more or less constant or even decreased. There were only a few cloudless days. It was usually more or less overcast with a variety of light winds. Southerly winds were generally associated with cool temperatures, while north easterly winds were associated with warmer temperatures.

Various effects of weather on activity and other aspects of rhino behaviour were to be expected (e.g., not surprisingly, temperature seemed to affect activity level). Another expected effect was that of wind on the smelling of the faeces sample. On November 16, 2005, heavy spray urinating had already started by about an hour before Cyrano actually sniffed the sample (Figure 3.12) and with Cyrano still not close to sample. However, during this day the wind freshened up and turned from west to south and brought the smell to the position where the bull was located, after which Cyrano started frequent smelling on the ground, scanning and spray urinating. Afterwards Cyrano went several times down and upwind until finally arriving at the sample. Unfortunately, this long delay between the start of spray urinating and smelling of the sample was rather unique. The same is true with the wind condition, namely that it turned in the right direction during the observation session. In most cases, the rhino found the sample within a few minutes after initiating spray urinating.

A comparison of the spray urinating and weather parameters during the test with the Christchurch sample CHC is shown in Fig. 3.26. While there seemed to be a trend similar to that in other tests for some parameters (e.g., cloud coverage or wind direction), there were also weaker or even opposite effects. On the whole, there was no clear dependency of behavioural activity on weather parameters.

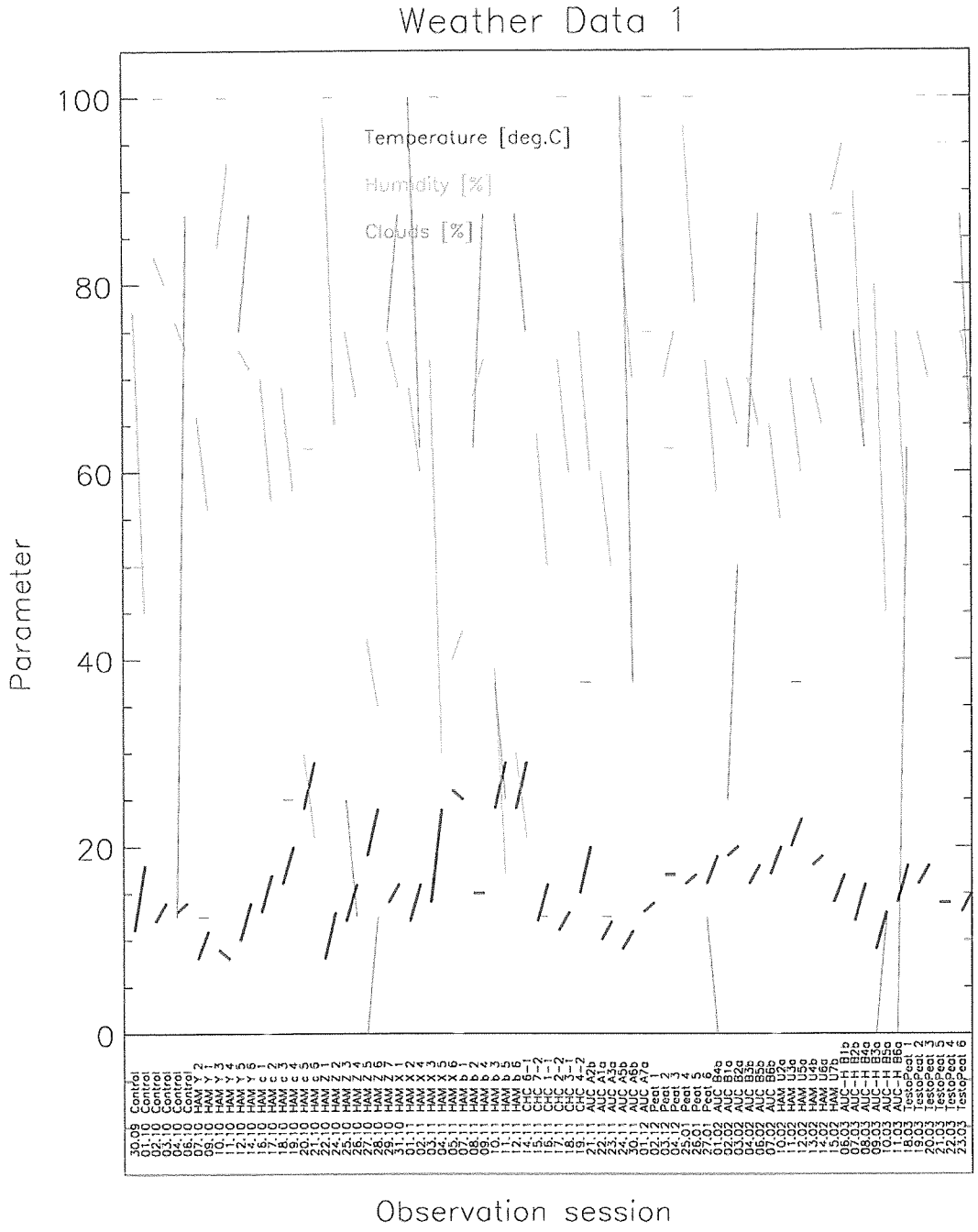

Figure 3.25 a. Weather data for observation days. Per session two values were recorded: at 9:00 AM and at 12:00 noon. Both values are connected by a line. Parameters are temperature (deg. C), humidity (%), and cloud coverage (%). At the bottom the sessions dates and corresponding sample names are given.

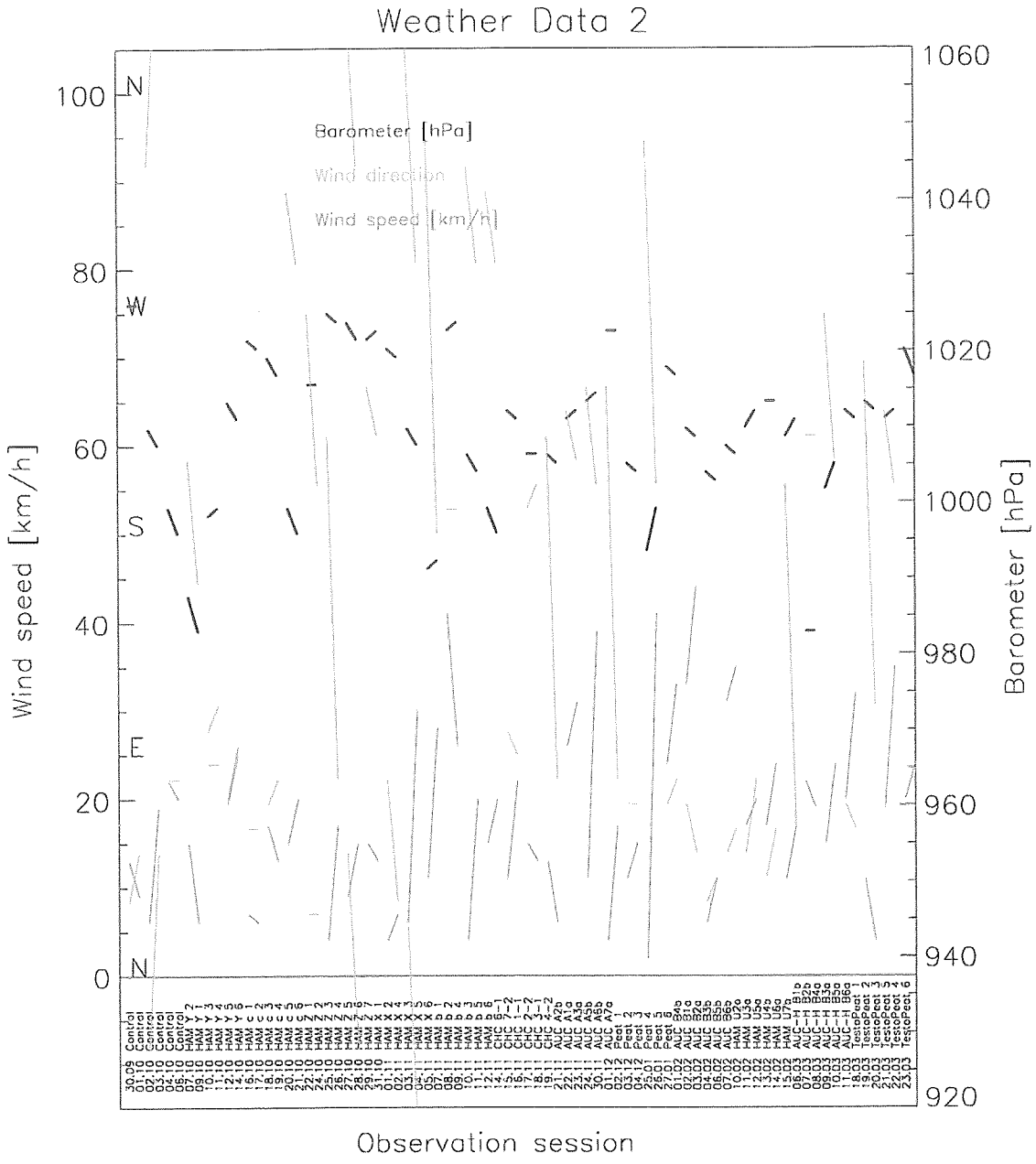

Figure 3.25 b. Weather data for observation days. Per session two values were recorded: one value at 9:00 and the other at 12:00. Both values are connected by a line. Parameters are barometric pressure (hPa, right scale), wind direction (left inner scale), and wind speed (km/h, left scale). At the bottom the session dates and corresponding sample names are given.

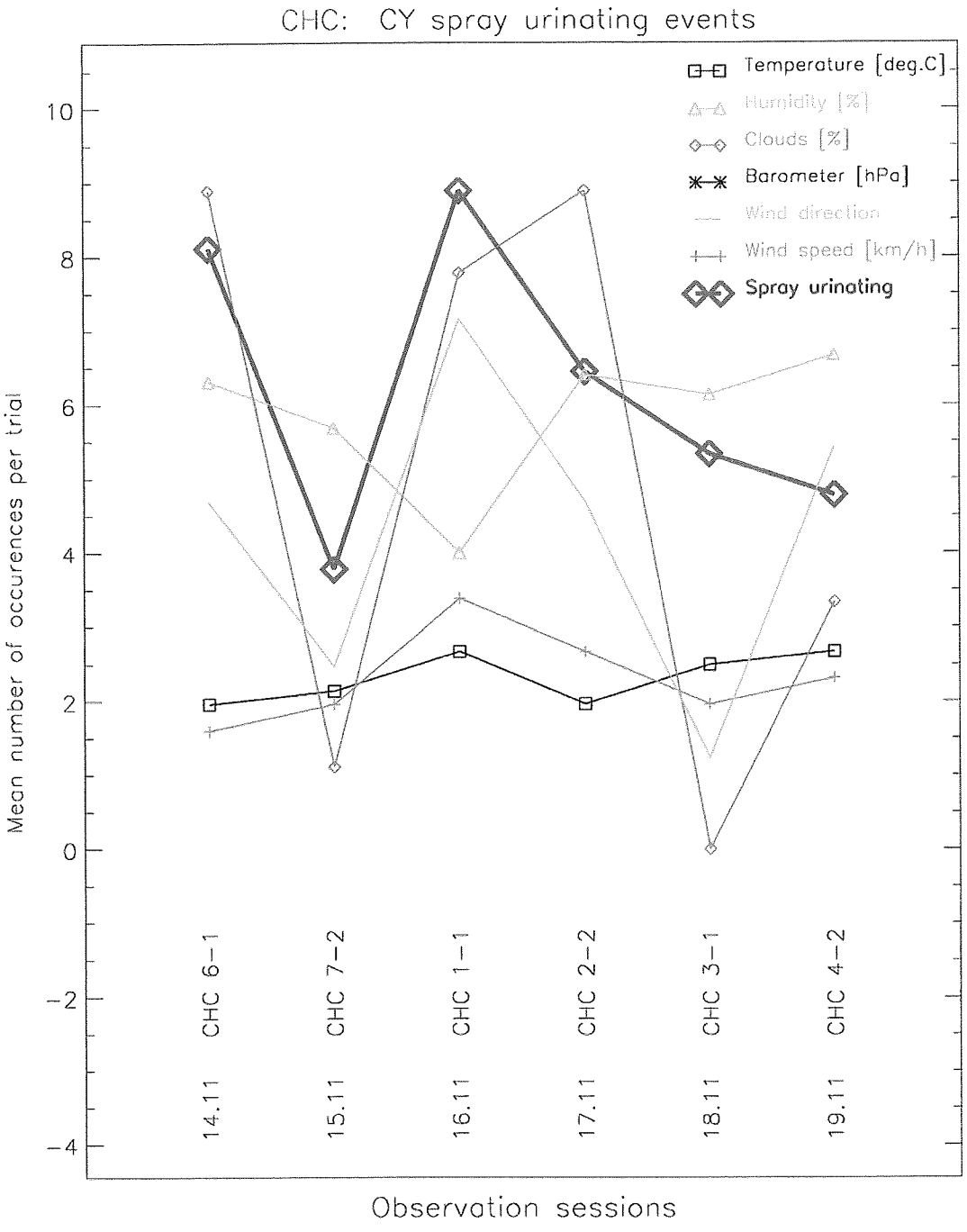

Figure 3.26. Comparison of the spray urinating activity (red diamonds) and weather parameters (see legend) for the CHC sample. Weather parameters are in relative units.

Chapter 4

4. Discussion

Corresponding to my two primary objectives (see the Introduction), there were two primary outcomes from this study: (1) experimental evidence that faeces from unfamiliar white rhinos carry information that influences the behaviour of adult rhinos in a zoo habitat, and (2) identifying olfactory constituents of the faeces that potentially stimulate these changes in behaviour.

4.1. Animal responses to foreign dung samples

White rhinos are known to be territorial with poor eyesight and a tendency to rely strongly on olfactory communication, and they routinely mark their territories with urine and faeces (Owen-Smith, 1973). My experimental subjects were one male and two female rhinos at Orana Wildlife Park in Christchurch.

First I demonstrated the vigilance of territorial bulls increased when they find foreign faeces in their territory. Cyrano and Utani showed the strongest reactions when smelling the samples of CHC, AUC A, and AUC B. They also spent significant time smelling the sample from HAM Y, HAM Y being the current breeding female at Hamilton Zoo. This result suggests that rhinos are able to recognise potential breeding partners or competitors. After Cyrano found the foreign sample, he smelled the ground over three times more often than the females. All rhinos became more active smelling the ground when male samples were presented, suggesting that the rhinos were stimulated by the odour from the foreign sample and were searching for more traces of a possible competitor or a mating partner. Another study (Kretzschmar 2002) has suggested that

white rhinos recognise testosterone in faeces and that detection of testosterone reveals to the rhino the potential proximity of a male intruder. I found support for this hypothesis from my testing with the TestoPeat sample. This sample contained testosterone and it evidently stimulated the smelling-the-ground response by Mapenzi and Cyrano.

Besides enhanced sniffing the ground, Cyrano also reacted to the faeces of foreign males by increasing how often he scanned. This suggests that, having detected evidence of a potential opponent being close by, Cyrano's general alertness was elevated.

In the wild, white rhino females tend to associate with peers of the same sex and age, they are tolerant of orphaned juveniles and they tend to live in stable herds of females and juveniles of up to six rhinos (Estes 2004). My findings were consistent with the literature, as I saw no conspicuous reactions by the females to the foreign-male faeces, although there was some evidence that they reacted to faeces from foreign juveniles and females.

Previous studies on free-living and on captive populations of white rhinos (Aberham, 2001; Kolar, 2002) have shown that discovering foreign faeces in their territory stimulates increased marking by territorial bulls. Consistent with this, I found that faeces from foreign males influenced the marking behaviour of white rhino males. Of all behavioural categories observed in my study, the enhanced spray urinating activity by Cyrano in response to male samples was the most dramatic and clear-cut response to the test samples. Even in the absence of a sample, marking his territory (the entire enclosure) was routine for Cyrano. When faeces from foreign males were placed in his enclosure, Cyrano dramatically increased how often he spray urinated. His reaction to male faeces was significantly higher than his reaction to faeces from females and juveniles. Spray urinating occurred in a sequence of three short bursts. Cyrano reacted to male samples with several of these sequences within a short time. After a fairly sudden onset of spray urinating activity, spraying frequency tapered-off and, within an hour, was back to his normal frequency. Evidently, Cyrano habituated to the stimulus.

Location of marking was also of interest, with most spray urinating by Cyrano occurring in the area where the faecal sample was located and close to the entrances of the enclosure, but Cyrano was not observed covering the sample. Cyrano's behaviour was similar to what has been called 'overmarking' in previous studies (see Aberham, 2001), this appears to be a mechanism by which

a male replaces a potential rival's scent with his own scent. This may function as a way of ensuring that the resident maintains his identity as the territory holder, enabling him to monopolise access to a group of females residing within his territory boundaries (see Gosling and McKay, 1990). The increased frequency of spray urination in response to male samples was accompanied by increased frequency of foot dragging by Cyrano. Foot dragging probably also functions in marking the territory.

An earlier study (Ketzschmar, 2002) has shown that, for white rhino males, territory marking and mating success are closely related. That is, free-living female rhinos are more likely to conceive from males that occupy the territories in which the females spend most of their time instead of from the males that have territories that they only briefly visit. This suggests that a male's mating success in nature depends critically on the male's ability to patrol, mark and defend a territory.

I was also interested in whether the presence of foreign faeces enhanced movement, stimulated rhinos to cluster or stimulated rhinos to synchronize their behaviour. On the whole, my findings imply that white rhino respond to foreign faeces in multifaceted ways that have not been reported before.

In nature, rhinos tend to become more active in the presence of foreign faeces. (Eisenberg & Kleinman, 1972). My findings were consistent with this. Agitation was perhaps the most basic response I recorded from white rhinos to foreign faeces, with the primary manifestation of this being increased frequency of walking. Agitation was especially noticeable when the samples came from foreign males. The effect on Cyrano was the most pronounced, but there was also evidence of agitation by the female subjects. Once agitated, Cyrano reduced his feeding activity, spending more time walking, scanning, and smelling the ground. The function of becoming agitated might be searching for the origin of, or avoiding the potential hazard from, the foreign animal that may be present.

Kolar et al. (2002) showed that rhino females respond strongly to the introduction of foreign urine marks from males. However, in my study, feeding and movement responses by females to the foreign samples were minimal. The females remained rather inactive, displaying little reaction to the different samples that I used for testing. Why my findings differ from the findings of Kolar et al. (2002) is not known.

Conradt & Roper (2000) proposed that segregation by sex-age class tends to be profitable because different sex-age classes tend to allocate time differently to different activities. Segregation and asynchronous behaviour may be favoured because it permits optimal time allocation by group members. This hypothesis may have applied in the observations I made at Orano Park. The median distance between Cyrano and the two females was 6.3 body lengths, but there was interesting variation in this distance depending on the sample being tested. The largest distances were in tests with foreign male faeces, but distances were also large when testing with heated samples and with TestoPeat samples. Surprisingly, distances were also large when testing with faeces from juveniles. The distances were shortest during testing with the control, with peat and with faeces from females. However, the distances females maintained between each other (median, 2.5 body lengths) remained relatively consistent across tests and tended to be smaller than intersexual distances. This suggests that there was social segregation between rhinos of different sexes at Orano Park.

Finding larger distance between Cyrano and the two females than between females may be explained by Cyrano being pre-occupied with patrolling his territory, especially when there were experimental stimuli present that served as clues for the presence of potential competitors. The ultimate cause of this male-female difference in distance may be the different life-history priorities of males and females (males prioritizing territorial and aggressive behaviour; females prioritizing feeding).

In nature, adult males of the white rhinoceros are generally solitary, with close associating with a cow being typical only when a cow is in oestrus (Owen-Smith, 1975). In a zoo, associating with a cow or not is not so clearly under the male's control, and the findings from my study tend not to show clear evidence of voluntary associating behaviour between male-female pairs. Cyrano and Utani displayed synchronous behaviour for only 50% of the time, with this synchrony remaining consistent regardless of the sample that was present. There were similar findings for the pair Cyrano – Mapenzi.

Feeding was the most common synchronous behaviour. Each rhino spent about 70% of its time feeding, with the probability of seeing both feeding at the same time being close to 50% (0.7 x 0.7 = 0.49). A frequency of 50% suggests that these instances of feeding at the same time are only coincidental rather than instances off coordinated feeding.

Rands et al. (2003) suggested that, by reducing predation risk, synchronization of behaviour, especially foraging and resting, can be advantageous to the individuals in a herd. In nature, rhino females tend to form small herds (Estes, 1991), with protection (especially of young) from predators possibly making these small herds adaptive. My data from Orano Park revealed female-female synchrony (especially of feeding) was recorded 65% of the time, suggesting some sort of rudimentary herding behaviour by females.

Cyrano reacted to samples from the Christchurch and Auckland bulls much more strongly than to samples from the Hamilton bull. The different home social environments of the bulls might help explain this finding. The Hamilton bull was the only male that did not experience competitors. He lived in a stable environment with three cows and his offspring. There were no other bulls in sight or in hearing or smelling distance. It was different from the Auckland bull. He shared an outside enclosure with another bull on a rotating schedule (i.e., every two days, one bull got access to the display enclosure while the other bull stayed in the holding paddock). It appears likely that this arrangement would have been experienced by the bull as continuous potential for male-male competition. In Christchurch, the bulls never have eye contact or access to the same territory, but the two males were almost certainly aware of each other by their senses of smelling and hearing. These different environments of males may have affected the chemistry of their faeces, with different chemistry in turn influencing Cyrano's different reactions to faeces.

Weather was probably not a serious confounding variable in the study. The weather parameters recorded were temperature, relative humidity, cloud coverage, wind speed and direction, and barometric pressure. While wind and increased temperature may have enhanced the carriage of the sample's odour, no clear dependency of behavioural activity on weather parameters was found.

4.2. Candidate signalling compounds

For understanding the various factors, including rhino age, gender, health and dominance status, that potential influence the olfactory components present in faeces, an important first step is the identifying of volatile components and determining the components that elicit strong reaction. With faeces, the distinction between hormones and pheromones becomes blurred. Hormones are, by definition, chemical signals that function within the body of a single individual, whereas

pheromones function as signals passed between different individuals. However, hormones (e.g., testosterone) excreted with faeces can potentially influence the behaviour of another individual and, thereby, qualify also as a pheromone. Other chemicals that may be common in faeces, including terpenoids and ethyl esters of fatty acids, may be reasonably envisaged as metabolic side products and yet be active in influencing the behaviour of other individuals, thereby functioning as pheromones.

Androgen concentrations of male white rhinos are known to be subject to seasonal trends, to appear in faeces and to be related to the mating success, attractiveness and status of males (Kretzschmar, 2002). Similar findings are known from studies of the African buffalo (Brown et al., 1991), plain zebra, Grevy's zebra (Chaudhuri & Ginsberg, 1990), wild dog (Montfort et al., 1997), and African elephants (Rasmussen & Schulte, 1998).

In this study, Cyrano's spray urinating activity did not appear to correlate with the testosterone concentrations in the faecal samples he was exposed to during testing. The rationale for making TestoPeat was to simulate the testosterone level of a 'super bull'. HAM U had the highest testosterone concentrations, this being comparable to the concentration of the 'super bull'. Surprisingly, no increase in activity was detected in tests using these two samples, and yet samples with medium concentrations (around 40 ng/g) did seem to cause an increased performance of territorial behaviour. These findings are of interest in the context of Kretzschmar's (2002) results from studying female white rhinos. Finding that variation among territorial males in the concentrations of testosterone and its metabolites did not necessarily influence a female's choice of mate, she concluded that the frequency of scent-marking by males showed only slight variations throughout the year and was independent from seasonal changes of androgen secretion. Yet I found that higher testosterone level in faeces did cause an increase in spray urinating by Cyrano, although not a linear increase. My findings suggest that perception of testosterone level, together with detection of some other compound or compounds, acted as signals for Cyrano indicating that a potential competitor male might be invading his territory.

Candidate compounds for this synergistic signalling system were found in male faeces: ethyl esters of low molecular weight, fatty acids (propanoic, butanoic, and pentanoic acids) that are volatile and have a distinct smell that can easily be recognized.

Anaerobic bacteria of the genus *Propionibacterium* produce propanoic acid (CH_3CH_2-COOH) as the end product when metabolising fatty acids containing odd numbers of carbon atoms, and also when metabolising certain amino acids. These bacteria are commonly found in the stomachs of ruminants, and their activity is partially responsible for the odour of Swiss cheese and of sweat.

Butanoic acid ($CH_3CH_2CH_2$-COOH) is another end-product of a fermentation process (discovered by Louis Pasteur in 1861) performed solely by obligate anaerobic bacteria. Mammals with good scent detection can smell butanoic acid (e.g., dogs detect it at10 ppb, but humans detect it only in concentrations above10 ppm). Detection sensitivity for rhinos is unknown (Anonymous, 2006).

Pentanoic acid ($CH_3CH_2CH_2CH_2$-COOH), like other low molecular weight carboxylic acids, has a very unpleasant odour. It is found naturally in valerian (*Valeriana officinalis*), a perennial flowering plant.

Carboxylic acids are organic acids characterised by the presence of a carboxyl group, which has the formula -C(=O)-OH, usually written as -COOH. The simplest series of carboxylic acids are the alkanoic acids, R-COOH, where R is an alkyl group.

An ester is the product of a condensation reaction between an acid (usually an organic acid) and an alcohol. Esters participate in hydrogen bonds as hydrogen-bond acceptors, but they cannot act as hydrogen-bond donors. Ester molecules, being unable to form hydrogen-bonds to each other, are generally more volatile than carboxylic acids of similar molecular weight.

Esters of fatty acids, like propanoic, butanoic, or pentanoic acid, occur in animal fats and plant oils. The aromas or tastes of low-molecular-weight esters of fatty acids, such as butanoic acid ethyl ester tend to be pleasant. However, hydrolysis of the ester occurs over time, resulting in liberation of fatty acids and, in the case of rhino faeces, could possibly create a unique time-dependent signalling mechanism. That is, the hydrolysis reaction results in the accumulation of a foul odour, which could be used by the rhinos to determine the relative age of the faeces. This, in turn, could carry information about the relative immediacy of a potentially threatening male presence.

The concentrations of the ethyl ester compounds in male faeces correlated positively with the spray urinating activity of Cyrano. While this was modelled by a linear fit, it could also be interpreted as a step function above which the urinating behaviour was triggered. Therefore, ethyl esters of low molecular weight fatty acids are prime candidates for the faecal constituents that enhance territorial activity in male rhinos.

Terpenoids, also known as isoprenoids, are a large and diverse class of naturally occurring organic lipids similar to terpenes. These are derived from five-carbon isoprene units (CH2=C(CH3)CH=CH2) assembled and modified in thousands of different ways. Most are multicyclic structures which differ from one another, not only in functional groups, but also in their basic carbon skeletons. These lipids are widespread in many kinds of organisms, including New Zealand plants (e.g., manuka, kanuka, and various low growing herbs) which are eaten regularly by rhinos in New Zealand zoos. Terpenoids contribute to the scent of eucalyptus, the flavours of cinnamon, cloves and ginger and the colour of yellow flowers. Well-known terpenoids include citral, menthol, camphor and the cannabinoids found in the Cannabis plant. Plant terpenoids have been extensively used by the food industry for their aromatic qualities. In animals, terpenoids form the precursors of steroids and sterols and are added to proteins to enhance their attachment to the cell membrane; this is known as isoprenylation.

Although terpenoids are used by insects for signalling (Harrewijn et al., 2001), vertebrates appear to make little use of terpenoids as signalling compounds. Consistent with this, I found no strong correlation between terpenoid concentration and behavioural reactions in the present study.

I found significant quantities of toluene in the samples. Toluene (C_7H_8), also known as methylbenzene or phenylmethane, is a clear, colourless, water-insoluble liquid with a strong, sweet, and pungent odour. It occurs naturally in crude oil. Toluene is also used to boost the octane rating of gasoline.

The name toluene was derived from an older name, 'toluol'. 'Toluol' refers to tolu balsam, an aromatic extract from the tolu tree (*Myroxylon balsamum*). This tree is native to the tropics in South America and has never been introduced to New Zealand (Dave Kelly, private communication). Although rhinos might have picked up toluene from eating contaminated food or water or from having skin contact with products that contain toluene (e.g., kerosene, heating

oil, paints, and lacquers), a more likely source is the breathing air contaminated by automobile exhaust.

4.3. Conclusions and Outlook

Free-living male rhinos signal their presence and their status to potential mating partners and opponents by marking their territories with urine and faeces. Here I've shown that even second-generation zoo animals do the same when they are stimulated by foreign faeces. From correlating territorial behaviour with faecal compounds, I have identified possible signalling components of the rhino's faeces. These include esters of fatty acids and perhaps testosterone and terpenoids.

Even simulated dung samples consisting of peat impregnated with potential signalling compounds initiated some response. This might have potential for environmental enrichment for rhinos in zoos. Using simulated rhino dung is likely to be considerably more economical than transporting actual dung between zoos.

For improving breeding success of white rhinos in captivity, a particularly promising method is having more than one bull within a zoo in completely separate enclosures and to rotate these bulls' access to the females. This method has been successful at the Werribee Open Range Zoo in Melbourne (Brooke Squires, personal communication), and it has also shown promise at Orano Park in Christchurch. Six weeks after my research ended, Cyrano was exchanged with the second Christchurch bull, and mating was observed. However, this method is expensive, as it requires having more than one bull in the zoo.

The rationale for my research was partly an interest in whether male odour is an effective surrogate for actual males. Determining whether this 'matchmaker' signalling mechanism will actually work for white rhinoceros was beyond the scope of my research, but my findings suggest that it is a viable hypothesis for future research.

However, there may be other benefits besides enhanced breeding success. For example, an aim with environmental enrichment is to improve the animals' quality of life rather than just to improve breeding success. Enrichment, for example, might increase physical activity and stimulate other natural behaviour. Benefits for the animals could potentially include less prevalent performance of repetitive stereotypical behaviour. In order to achieve a continued

benefit, care must be taken to ensure that novelty is maintained. Sustaining an animal's interest may require a random or rotating schedule of enrichment (Shepherdson, 1998). That enrichment has powerful effects on zoo animals is perhaps not surprising. After all, the natural world is constantly changing, forcing animals to adapt to new situations. A relatively unchanging zoo environment may be very unlike the world in which the species evolved. In a zoo, proving novelty as part of an enrichment protocol potentially goes a long way toward simulating the variability of the natural world.

This study suggests that scent from foreign faeces has multifaceted behavioural effects of captive white rhinoceroses and that the volatile components responsible for these effects are identifiable. For a rhino, the features of the world that are salient tend to be especially factors related to olfaction. Moving novel odour from foreign dung, and perhaps even by using artificial dung, we may have an economical way to enrich the olfactory environment of the white rhino.

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