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Abstract: The goal of this project was to document the responses of free-ranging cheetahs (*Acinonyx jubatus*) and other large African felids to novel scents in an attempt to refine methods for surveying felid populations. Specifically, the purpose of the study was:
1) To ascertain whether African felids are attracted to novel scents. While captive cats are drawn to a wide variety of fragrances, we wanted to assess the response of free-ranging felids to novel scents where they might i) explore scents because they are unfamiliar and interesting, or ii) avoid scents because they might be associated with human activity.
2) Assess whether these scents would elicit rubbing responses that could be used to facilitate the collection of hair samples from African felids. If successful, this technique could be used as an effective tool to non-invasively collect hair samples for genetic analyses.

Using Scent Attractants to Non-Invasively Collect Hair Samples from Cheetahs, Leopards and Lions

By
Patrick Thomas[1] PhD, Guy Balme[2], Luke Hunter[1], and Joan McCabe-Parodi[1]*
[1] Wildlife Conservation Society, USA
[2] MunYaWana Leopard Project, RSA
** author for correspondence, email: pthomas@wcs.org*

Introduction
Many species of carnivores will rub against inanimate objects (Reiger, 1979), and many but not all species of felids will cheek rub against objects or conspecifics (Mellen, 1993). Cheek rubbing deposits scent (Bradshaw and Cameron-Beaumont, 2000), and can serve as a visual or olfactory means of marking territory (Smith et al., 1989), picking up scent (Wemmer and Scow, 1977) and providing information on reproductive status (Foster, 1977). Male domestic cats, for example, can assess the reproductive status of females from their cheek gland secretions (Verbene and DeBoer, 1976).

In zoological parks, the application of various novel scents, especially perfumes and colognes, has been used to provide behavioral enrichment by attracting felids, encouraging exploratory behavior and eliciting cheek rubbing behavior (Williams et al., 1999; Mellen et al., 1998; Calderisi, 1997). Captive felids typically show strong responses to novel odors in their environments. The reasons for such behavior are not well known but may include over-marking to reaffirm ownership of their territory, gleaning chemical information from the scent, or alleviating routine. In the field, scents applied to hair traps have assisted biologists in attracting felids to collect hair samples for DNA analyses from Canada lynx (*Lynx canadensis*) (Turbak, 1998) and ocelots (*Leopardus pardalis*) (Weaver et al., 2003).

The goal of this project was to document the responses of free-ranging cheetahs (*Acinonyx jubatus*) and other large African felids to novel scents in an attempt to refine methods for surveying felid populations. Specifically, the purpose of the study was:

1) To ascertain whether African felids are attracted to novel scents. While captive cats are drawn to a wide variety of fragrances, we wanted to assess the response of free-ranging felids to novel scents where they might i) explore scents because they are unfamiliar and interesting, or ii) avoid scents because they might be associated with human activity.

2) Assess whether these scents would elicit rubbing responses that could be used to facilitate the collection of hair samples from African felids. If successful, this technique could be used as an effective tool to non-invasively collect hair samples for genetic analyses.

Methods
To assess the efficacy of both attracting cheetahs to scents and whether hair traps collected a sample suitable for DNA analysis, we first conducted trials with captive cheetahs in the Bronx Zoo. Following those trials, we tested the responses of wild cheetahs (as well as leopards and lions) in situ in South Africa.

We experimented with a variety of commercially available perfume and colognes applied to hair traps. Hair traps were modified from commercial slicker-type dog grooming brushes with stainless steel wires. The handles were removed from brushes, and the pad and wires were nailed to both horizontal and vertical structures within the cheetahs' exhibit.

Zoo Study:
This study involved the Bronx Zoo's pair of cheetahs. The animals (one male and one female

captive-born siblings) were 11 years old at the onset of the project. The study was conducted in their 0.1 ha, outdoor naturalistic enclosure. Twenty-four different perfumes and colognes previously tested on the zoo's Amur tigers, *Panthera tigris altaica* (n = six animals) and snow leopards, *Uncia uncia* (n = 14 animals) were utilized during the study. Each day, three scents were placed in different locations in the cheetahs' exhibit prior to their release into the exhibit from night quarters. Scents were sprayed on both horizontally and vertically oriented objects. The animals' behavior was recorded for 0.5 hr immediately after the cheetahs were given access to the exhibit. Behavioral data collected included: latency to inspect the scent, number of visits to the scent, contact/interaction time with the scent, and behavior at the scent. Data was collected for 24 non-consecutive days, and the cheetahs were exposed to each scent on three different days. Specific scents were never tested against each other on more than one day.

Field Study:

The field trial took place at the Phinda Private Game Reserve, in northern KwaZulu-Natal, South Africa (27°48'S, 32°19'E) in October 2003 (Figure 1). Phinda comprises 14,500 ha (33,350 acres) and measures approximately 42 km long by 3-5 km wide. It contains several distinct habitats including a dry sand forest, savanna, closed mixed bushveld, closed red sand bushveld, dry mountain bushveld, open mixed bushveld, open red sand bushveld, palmveld, and riparian floodplains. At the time of the study Phinda had a small population of cheetahs (n ≈ 8), two lion prides (n = 15 animals), and a population of resident and transient leopards. Lions and cheetahs at Phinda have been intensively monitored since 1992 (Hunter 1998) while leopards are the focus of an ongoing research effort in which individuals are monitored by camera-trapping and telemetry (Hunter *et al* 2003, Balme & Hunter *in press*).

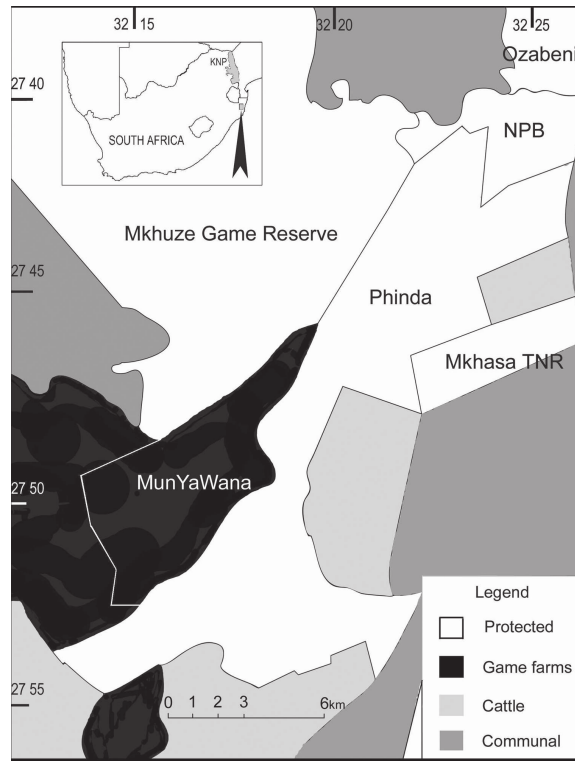

Fig. 1. Location of the field study site, Phinda Private Game Reserve, South Africa

Fifty hair traps were placed throughout the reserve on both horizontally and vertically oriented objects and baited with one of seven scents (Figure 2). The traps were located at the intersections of vehicular trails through the reserve that cats frequently followed. Traps were also placed near specific trees or elevated areas known to be utilized by cheetahs. Traps were checked and re-scented every two to three days. Whenever a cat rubbed against a hair trap, the trap was removed, temporarily stored in a manila envelope and a new trap installed in its place. Hair was removed from the traps using stainless steel forceps and stored at room temperature in small manila envelopes. The hair samples were initially identified by species from tracks at the trap station; later this was confirmed by analyzing the hair samples under microscopy against a reference collection.

We also conducted opportunistic trials (n = 8) whenever we encountered cheetahs, leopards (P*anthera pardus*) or lions (*Panthera leo*) in the field. We tested a pair of cheetahs on two occasions, five lions

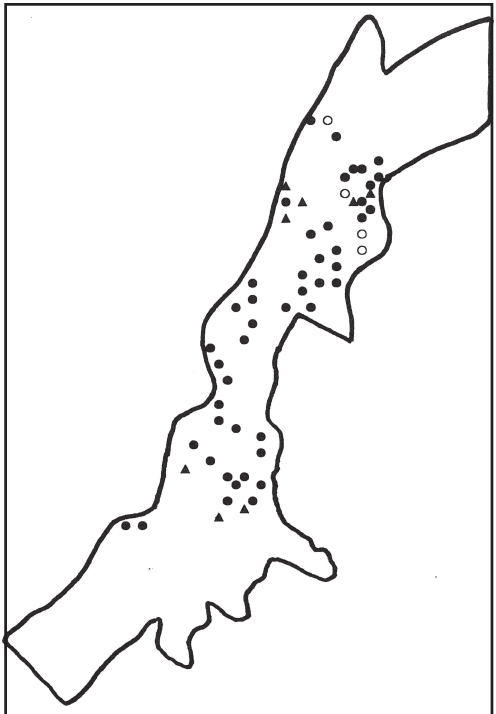

Fig. 2. Location of hair traps within the Phinda Game Reserve (●), traps where hair samples were collected (○), and sites where felids were opportunistically encountered and tested. (▲).

on two occasions, 10 lions on one occasion, single leopards on two occasions and a female leopard with two adolescent cubs on one occasion. We conducted these trials when we located animals that were at rest or moving in a predictable direction. When animals were at rest, we drove slowly towards them from an upwind direction to move as near to them as possible without disturbing them. We sprayed a tree trunk, shrub or log with a scent upwind of them, and retreated at least 50m to observe the animals' behavior for a minimum of 20 min. When animals were moving in a predictable direction, we drove well ahead of the cats, sprayed a scent on a piece of prominent vegetation, and moved off. We observed the cats' behavior as they passed the sprayed object until they had walked at least 100m past it.

Results

Zoo Study:

The cheetahs at the Bronx Zoo investigated the vast majority of the scents that were offered to them within the first 0.5 hr that they were given access to the exhibit (Table 1). Each cheetah inspected 21 (87.5%) of the 24 scented sites, with only one scent being ignored by both animals. On average it took the male 12 min (sd = 8.7) to come in contact with a scent, while it took the female 10.9 min (sd = 8.5) to locate an area that was scented. The cheetahs typically did not locomote in tandem and came across the scents independently. At no time during the study did the two cheetahs investigate the same scent at the same time.

There was a considerable difference in the cats' responses to the various scents once they were encountered ($X^2_{(23)} = 35.0$, $P < 0.05$). While nearly all the perfumes and colognes were investigated, only seven (29.2%) of the 24 scents elicited a powerful rubbing response (Figure 3). The cheetahs showed no preference for rubbing against vertical or horizontal structures. On occasion the cheetahs would spend nearly half of the sampling period rubbing against an object that had been sprayed with a particular scent. In general, however, the cats explored most (> 70%) of the scents for less than one minute, and in the majority of instances their responses consisted of simply sniffing the object that had been sprayed with the scent. There was not a significant difference in the amount of time the male and female cheetah spent investigating the scents ($t_{(23)} = 0.66$, $P > 0.05$). More than half (54%) of the scents were investigated on more than one occasion during the sampling period, with both cats exhibiting similar exploratory rates ($T_{(23)} = 151$, $P > 0.05$).

The hair traps proved to be very effective in collecting and retaining hair, with traps retaining significant amounts of hair for at least 48 hours even when they were exposed to heavy rain.

Field Study:

Fifty hair traps were set out in the reserve for a total of 864 trap days over a three-week period. Figure 2 shows the locations of the traps within the reserve, the sites where hair was collected from a trap, and the locations where felids were encountered and opportunistically tested. Four traps, baited with three different scents, collected hair samples from big cats: one from a lion and three

from leopards. No hair samples were collected from cheetahs. Hair samples were collected from traps that were attached to both horizontal and vertical structures but we did not have sufficient sample size to test for differences.

Although lions and leopards were fairly equally distributed throughout the reserve (Hunter 1998, Balme & Hunter unpubl data), the four traps that collected hair samples were within approximately five km of each other, in the northern part of the reserve. The hair traps were also investigated by other species including African elephants (*Loxodonta africana*), spotted hyenas (*Crocuta crocuta*), warthogs (*Phacochoerus aethiopicus*), and bushpigs (*Potamochoerus porcus*).

Only one of eight opportunistic tests produced an observable behavioral response, from a female leopard that was at rest when located (Table 2). After spraying a tree approximately 50 m from her, she got up, walked directly to the tree, and sniffed the spot that had been sprayed. Almost immediately she sneezed several times and left. She did not cheek rub the site. In none of the opportunistic trials did any of the species deliberately move away or avoid an area that had been sprayed with a scent.

Discussion

The zoo study documented that cheek rubbing was part of the cheetah's behavioral repertoire, and that perfumes and colognes could elicit this behavioral response. Subsequent studies on the zoo's lions and leopards confirmed that these species (as well as the zoo's Amur tigers and snow leopards) also engaged very frequently in this behavior (Thomas, unpubl data). The zoo study also confirmed that both sexes of cheetahs cheek rubbed against inanimate objects, and that they were attracted to many of the same scents (although there was some individual variability).

The field study showed that certain perfumes and colognes elicited cheek rubbing behavior in free-ranging African felids, although their rate of response was dramatically lower than what was observed with captive animals. While the behavior of the cats indicated that they were not alarmed by the scents and they did not actively avoid them, their response was at best ambivalent. Even adolescent lions and leopards, which might be expected to be more inquisitive, largely ignored freshly deposited perfumes and colognes. This is surprising given the natural curiosity of felids towards novel items and the dramatic responses recorded in captive individuals. One possible explanation is that cats in the wild are presented with a such a wide range of stimuli that novel scents are not worth investigating unless they are associated with conspecifics, food or other more 'relevant' factors. We did not assess the responses of cats to other potential attractants such as blood or the urine of unfamiliar conspecifics so this remains speculative, though previous successes with lynx and ocelot used a scent mixture based on the smells of food (Turbak, 1998; Weaver et al., 2003). Alternatively, perhaps the scents we used were not novel enough to the wild individuals we tested. All individuals in our study were highly habituated to the presence of people (chiefly, tourists in vehicles) and their various smells including, presumably, perfume and cologne. As unlikely as it seems, it is possible that the scents we tested were ignored because they were considered familiar.

The lack of success collecting samples from cheetahs may be related to the fact that their population in Phinda was low during the time of the study. In particular, there were no adult male cheetahs present who, being territorial, may be more likely than other cohorts to respond to a novel scent. There was no direct evidence (e.g., presence of tracks around a trap station) that they ever encountered hair traps, although their behavioral responses during opportunistic testing were not obviously different from those of lions and leopards.

Further work should evaluate whether other scents can effectively induce African felids to cheek rub to collect hair samples for genetic analyses. We recommend a similar two-stage trial process (i.e. on captive animals followed by in situ trials) employing a variety of scents that might reasonably be expected to hold greater interest for wild cats. In particular, smells indicating a possible food source or conspecific might hold greater promise.

Scent	Mean Latency to Inspect Scent (min)		Mean Number of Visits to Scent		Mean Contact Time with Scent (sec)		Behavior (+ = rubbing response)	
Allure	-	13	-	0.3	-	2		
Altitude	17	7	0.7	0.7	4	6		
Beautiful	9	14	0.3	0.3	2	2		
Charlie	7	4	2.0	1.7	13	18		
Ciara	5	8	1.3	2.3	66	61	+	+
Claiborne Sport	22	9	1.0	1.3	8	8		
Crave	-	-	-	-	-	-		
D. Cool Water	18	11	0.3	0.3	5	0.7		
Dolce Gabonna	8	-	0.3	-	4	-		
Drakkar Noir	3	4	2.3	2.0	167	204	+	+
Dunhill Desire	7	10	1.0	1.7	6	18		
Eau Arpege	13	9	0.7	0.7	2	3		
Escape	15	7	1.0	1.0	17	150		+
Eternity for Men	8	5	0.7	1.3	3	14		
Farenheit	12	16	1.3	0.3	9	2		
For Her	16	-	0.7	-	3	-		
Kouros Cologne Sport	-	19	-	1.3	-	7		
L'Air du Temp	3	2.5	2.7	2.3	451	794	+	+
Mainbo	7	5	1.7	2.0	18	26		
Obsession for Men	5	2	2.7	2.0	464	873	+	+
Oscar	9	11	0.3	1.7	7	22		
Paco Rabonne	6	5	2.3	1.7	233	181	+	+
Samsara	4	7	1.7	0.7	40	9	+	
Ultravioleet Man	3	3	2.0	1.7	48	37		

Table 1. Responses of Bronx Zoo cheetahs to various scents. Mean latency to inspect scent is rounded to the nearest minute, and mean contact time is rounded to the nearest second.

Trial	Location	Scent	Species	Reaction
1	27° 44' 324" S, 32° 22' 741" E	L'Air du Temp	1 leopard	sniffed & sneezed
2	27° 44' 687" S, 32° 20' 421" E	Obsession for Men and Paco Rabonne	5 lions	ignored scent
3	27° 44' 036" S, 32° 23' 883" E	Paco Rabonne	1 leopard	ignored scent
4	27° 45' 594" S, 32° 19' 996" E	Ciara	5 lions	ignored scent
5	27° 50' 675" S, 32° 17' 213" E	L'Air du Temp	3 leopards	ignored scent
6	27° 52' 314" S, 32° 18' 823" E	Ciara	2 cheetahs	ignored scent
7	27° 52' 363" S, 32° 18' 782" E	Obsession for Men	2 cheetahs	ignored scent
8	27° 44' 845" S, 32° 20' 902" E	Obsession for Men	10 lions	ignored scent

Table 2. Behavioral responses of African felids to opportunistic testing of various scents.

Acknowledgements

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Figure 3. Female cheetah at the Bronx Zoo cheek rubbing against a hair trap sprayed with perfume. *(Photo by Julie Larsen-Maher)*