

Snake harassment in the Cape ground squirrel (*Xerus inauris*): variation in anti-predator behaviours, predator discrimination and venom resistance in a facultative cooperative breeder.

by

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Abstract

Predator harassment is an anti-predator behaviour that may increase a harasser's risk of predation but decrease the potential for predation for other members of a group. The Cape ground squirrel (*Xerus inauris*) is a facultative cooperative breeder from southern Africa that harasses venomous snakes. The objective of my study was to examine predator harassment to determine: 1) whether predator harassment was part of alloparental care by comparing harassment behaviour among age and sex classes; 2) how individuals adjusted their behaviour against different snake species; 3) whether olfaction was used in discrimination of snakes; and 4) if individuals possessed venom resistance against venomous snakes. I found that females with juvenile offspring harass snakes longer and more intensely than other individuals, suggesting that predator harassment was a maternal behaviour. Squirrels increased harassment, inspection and vigilant behaviours with risk when exposed to both live snakes and snake odours suggesting they can use olfaction to discriminate snake predators. I also found no venom resistance in Cape ground squirrels concluding the cost of envenomation was significant.

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List of Tables

Table 2.1: Who in the social group is participating in predator harassment? Hypotheses, predictions and results for Cape ground squirrels.....	32
Table 2.2: Can they discriminate? Hypotheses and predictions for Cape ground squirrels.....	32

List of Figures

Figure 2.1: Comparing median mobbing duration in seconds among age/sex classes of Cape ground squirrels.....	33
Figure 2.2: Comparing median mobbing intensity among age/sex classes of Cape ground squirrels.....	34
Figure 2.3: Comparing median mobbing durations in seconds of Cape ground squirrels when exposed to different snake species.....	35
Figure 2.4: Comparing median mobbing intensity of Cape ground squirrels exposed to different snake species.....	36
Figure 2.5: Comparing median tail flagging frequency of Cape ground squirrels exposed to different snakes species.....	37
Figure 2.6: Median inspection duration in seconds of Cape ground squirrel social groups (means) exposed to different snake species.....	38
Figure 2.7: Cape ground squirrel social group mean proportion of the trial spent vigilant among treatments (arcsine transformation).....	39
Figure 3.1: Comparing time spent within 0.5 m of the mat during the trial and scent treatments by Cape ground squirrels.....	54
Figure 3.2: Comparing cube inspection frequency by Cape ground squirrels and snake scent treatment.....	55
Figure 3.3: Comparing harassment associated behaviour frequency in Cape ground squirrels with different snake scents.....	56
Figure 4.1: Diameter of area of gelatinase activity caused by pooled venom of puff adders (dark gray) and snouted cobras (light gray).....	67

Table of Contents

Abstract.....	ii
Acknowledgements.....	iii
List of Tables.....	iv
List of Figures.....	iv
List of Copyrighted Material for which Permission was Obtained.....	vi
Chapter I: Introduction.....	1
Chapter II: Anti-snake behaviour in a facultative cooperative breeder, the Cape ground squirrel.....	7
Introduction.....	7
Methods.....	13
Results.....	19
Discussion.....	22
Chapter III: Olfactory snake-predator discrimination in the Cape ground squirrel.....	40
Introduction.....	40
Methods.....	43
Results.....	47
Discussion.....	47
Chapter IV: No evidence for proteolytic venom resistance in southern African ground squirrels.....	58
Introduction.....	58
Methods.....	59
Results.....	61
Discussion.....	61
Chapter V: Conclusion.....	69

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Chapter I: Thesis Introduction

Predator-prey interactions are significant selective forces that shape many morphological, physiological and behavioural traits in both predators and their prey (Lima & Dill 1990). For predators to be successful, they need to encounter, detect, identify, approach, subjugate and consume prey (Sherbrooke 2008). Prey species can respond at multiple stages in the predation sequence to decrease their predation risk, reducing the possibility of encounter, detection, and capture. Some animals possess mechanisms to decrease predation even after capture (Caro 2005).

Some prey species approach and even harass their predators. These behaviours are known as predator harassment or mobbing. Predator harassment has been widely studied for its evolutionary function, as the behaviour appears to impose some costs, including energetic costs, opportunity costs (defined as the cost of time spent harassing that could be spent doing other biologically important activities such as foraging or resting) and the risk of injury or death (Dugatkin & Godin 1992). The costs and benefits of predator harassment are dynamic and an individual's decision to approach and harass is influenced by others in the group, their own reproductive interests and the type of predator encountered.

By comparing predator harassment variation *among* individuals in a group, mobbing has been used to determine which individuals benefit from, and therefore participate, in predator harassment. For example, differences in predator harassment between the sexes during nest defence have been examined to answer questions about sex differences in parental investment (Curio et al. 1985). Similarly, differences in

relatedness or familiarity among group members and cooperation in group predator harassment has been used to test kin selection and reciprocity-based hypotheses to explain cooperation in several species (Griesser & Ekman 2005; Krams et al. 2006; Krams et al. 2010). Also, by comparing closely related species' group harassment behaviour to their life history characteristics, group harassment behaviours have been used to formulate a hypothesis for the evolution of cooperative breeding (Doerr & Doerr 2006). The anti-predator tactics hypothesis predicts the benefits of group predator harassment will be greater than the costs in species that live in open habitats and have predators that can be deterred by harassment. Group harassment behaviour has been documented in some species of cooperative breeders, including many birds and mammals (Francis et al. 1989; Maklakov 2002; Arnold et al. 2005; Graw & Manser 2007).

The second chapter of my thesis examines predator harassment participation in a species that occurs in open habitats and encounters several species of predatory snake (Doerr & Doerr 2006). The Cape ground squirrel (*Xerus inauris*) is a southern African species of ground squirrel with an unusual social system living in a diverse predator community (Waterman 1995; Skurski & Waterman 2005; Unck et al. 2009). The Cape ground squirrel has been documented to approach and harass several venomous snakes though no detailed research has been conducted on predator harassment in this species (Waterman 1998; Waterman & Roth 2007). Cape ground squirrels are unique among sciurids because they are facultative cooperative breeders. Females live in closely related groups separated from adult males, which disperse and live in their own unrelated bands (Waterman 1995; Pettitt & Waterman 2011). The objective of the second chapter of my thesis was to examine the responses of Cape ground squirrels of different sexes and ages

to predatory snakes to determine whether snake harassment is attributable to benefits accrued via direct fitness (maternal or paternal care) or indirect fitness (alloparental care) in this cooperative breeder.

Predator harassment has also been used as a model to study many aspects of anti-predator behaviours such as predator discrimination because many harassment associated behaviours are easily observed (Hartley 1950). Prey trade-off anti-predator behaviours with other important activities, such as foraging, reproduction or resting. By adjusting their anti-predator response to reflect the magnitude of the threat they can decrease opportunity and energetic costs of harassing non-threatening species. Many species have displayed the ability to assess the degree of threat posed by a predator by decreasing or increasing the level of harassment directed at the predator, including many birds, mammals, and fish (Coss & Owings 1978; Helfman 1989; Ouattara et al 2009; Colombelli-Negrel 2010).

My second and third chapters examine how Cape ground squirrels change anti-predator behaviours among different snake species to determine if Cape ground squirrels can discriminate among their snake predators. Cape ground squirrels live in a diverse snake-predator community with many venomous and non-venomous snake species that consume mammals and represent various degrees of threat to Cape ground squirrels (Alexander & Marais 2007). For example, the puff adder (*Bitis arietans*) and the snouted cobra (*Naja annulifera*) are two highly venomous snakes that live sympatrically with Cape ground squirrels. Both species are potentially lethal to adult Cape ground squirrels; however, detection and avoidance would decrease the likelihood of capture. However, snouted cobras actively pursue more vulnerable juvenile squirrels in burrows while puff

adders are mainly sit-and-wait predators, making cobras more dangerous to juvenile squirrels in the social group (Alexander & Marais 2007; Graw & Manser 2007). My second and third chapters examine whether Cape ground squirrels are able to identify snake predators by sight as well as by olfactory cues and whether they treat the actively hunting cobra as more threatening than the puff adder.

Several species that harass venomous animals have been found to have venom resistance, including two species of North American sciurids (Biardi et al. 2000; Biardi & Coss 2010). Possessing venom resistance would greatly reduce the cost of snake harassment as it decreases the risks of injury or death from defensive bites from venomous snakes. My final chapter tests whether Cape ground squirrels have venom resistance like other sciurids that harass venomous snakes in order to understand the costs of snake harassment.

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Chapter II: Anti-snake behaviour in a facultative cooperative breeder, the Cape ground squirrel

Abstract

Predator harassment is an anti-predator behaviour that may increase an individual's risk of predation, as individuals approach, threaten and harass a potential predator, yet this behaviour is still not well understood. The Cape ground squirrel (*Xerus inauris*) is a highly social facultative cooperative breeder from southern Africa that harasses several species of venomous snakes. I examined whether harassment was part of alloparental care by comparing harassment behaviours among different age and sex classes in the Cape ground squirrel social group. I also assessed how individuals adjusted their behaviour dependent on levels of risk by examining the Cape ground squirrel's harassment behaviour among non-venomous, elapid, and viperid snakes. I found adult females with emerged juvenile offspring took the most risk, harassing for longer durations and at higher intensities, suggesting that snake harassment was a maternal behaviour. Individuals only harassed the highest risk elapid snake but increased vigilance and inspection with increasing snake risk suggesting that the Cape ground squirrel can discriminate between different types of snake predators.

Introduction

Anti-predator behaviours are influenced by many factors, including habitat, predator type, social structure and reproductive interests of the individual (Chandler & Rose 1988; Griesser & Ekman 2005; Doerr & Doerr 2006; Colombelli-Négrel et al.

2010). While passive forms of anti-predator behaviours are common (vigilance, crypsis, escape) some individuals approach and actively harass (mob) their predators (Caro 2005).

Predator harassment, or mobbing, is an approach towards a potentially dangerous predator, which is usually paired with vocalizations and visual displays (Curio 1978). Such harassment has been documented in a variety of taxa, including many birds, mammals and fish (Owings & Coss 1977; Berger 1979; Dominey 1983; Shields 1984; Gursky 2006) and is most likely multi-functional for most species (Maklakov 2002). Some hypothesized functions of predator harassment include encouraging the predator to leave (move-on hypothesis) (Curio 1978; Flasskamp 1994), recruiting other individuals to join the mob (Owings & Coss 1977; Tamura & Yong 1993), alerting others in a social group to a predator to increase group vigilance (Owings et al. 1986; Graw & Manser 2007), or communicating risk to offspring (cultural transmission) (Curio et al. 1978). Harassment behaviours seem to be most influenced by the type of predator (stimulus) as well as the presence (quantity, proximity, relatedness) of other group members (Ostreiher 2003).

Harassment of a potential predator is most likely a risky behaviour. Costs associated with predator harassment could include energetic costs and outright risk of injury or death (Dugatkin & Godin 1992). The ability to assess the degree of threat posed by a predator and to react with a graded response could decrease such risks (Patterson et al. 1980; Helfman 1989). Many species display discrimination when harassing predators, including both birds and mammals (Chandler & Rose 1988; Loughry 1989; Griesser & Ekman 2005; Colombelli-Négrel et al. 2010). North American ground squirrels, such as the black-tailed prairie dog (*Cynomys ludovicianus*) and the California ground squirrel

(*Otospermophilus beecheyi*), can distinguish between venomous and non-venomous snakes and treat venomous snakes with greater caution during harassment (Loughry 1989; Towers & Coss 1990).

Social structure and reproductive interests also influence predator harassment behaviour, as they affect which individuals benefit from, and therefore participate in, predator harassment. For example, Doerr & Doerr (2006) proposed an anti-predator tactics hypothesis to explain the evolution of cooperative breeding in Australasian treecreepers (Climacteridae). Benefits of group defence would be greater than the cost of conspicuousness in species that lived in open habitats and had predators that could be deterred by harassment. Group harassment behaviour has been documented in many species of cooperative breeders, including birds like noisy minors (*Manorina melanocephala*), Arabian babblers (*Turdoides squamiceps*) and Florida scrub jays (*Aphelocoma coerulescens*), and in mammals like suricates (*Suricata suricata*) and dwarf mongooses (*Helogale parvula*), (Rasa 1987; Francis et al. 1989; Maklakov 2002; Arnold et al. 2005; Graw & Manser 2007).

The objective of my study was to examine the importance of group vigilance and active defence in a species that occurs in open habitats where concealment-based anti-predator tactics would be less advantageous (Doerr & Doerr 2006). The Cape ground squirrel (*Xerus inauris*) is a southern African sciurid that occurs in open habitat, is highly social and actively harasses several species of snake (Waterman 1997). The Cape ground squirrel has also been classified as a facultative cooperative breeder though the role alloparents play in predator harassment has not been examined in detail (Waterman 1995; Waterman 2002; Pettitt & Waterman 2011). Juveniles, once emerged from the natal

burrow, can feed independently and do not require provisioning (one role alloparents can play), which makes Cape ground squirrels unique compared with previously studied cooperative breeders. However, predation is thought to be a major cause of juvenile mortality (Waterman 2002). I examined the role social group members played in the detection and active defence against snake predators.

Cape ground squirrels are exposed to a diverse snake predator community, more diverse than previously studied North American ground squirrels (Alexander & Marais 2007). Several ground squirrel species' have the ability to discriminate between one venomous and one non-venomous snake (Loughry 1989; Towers & Coss 1990); however, the more complex diversity of snakes that prey on Cape ground squirrels allowed me to test whether they can also discriminate between different venomous snakes. Cobras (Elapidae) are fast, actively hunting snakes and probably pose more of a threat than puff adders (*Bitis arietans*), which depend on the element of surprise to capture prey (Graw & Manser 2007). I examined whether Cape ground squirrels discriminated between two venomous snake species as well as between venomous and non-venomous snakes.

I examined snake harassment behaviour among sex/age classes in the Cape ground squirrel social group to test several hypotheses to determine whether snake harassment behaviour was part of alloparental or parental care. If predator harassment was part of alloparental care, I predicted individuals of all age/sex classes will participate in mobbing snakes with their social group (Table 2.1: Hypothesis 1). Alternatively, if predator harassment was a maternal behaviour then only mothers of vulnerable juvenile offspring (offspring that are less than 6 months old) would exhibit harassment behaviours

and these mothers would exhibit the most risky behaviours (by harassing for longer durations, and at higher intensities) (Table 2.1: Hypothesis 2).

I also tested whether Cape ground squirrels discriminated among different species of snake by examining how they changed their harassment and vigilant behaviours in response to differing threat levels. If Cape ground squirrels could discriminate between snakes that pose no threat to self or offspring (like a small non-venomous snake) and snakes that pose high risks (like a large venomous snake) then they would increase vigilance, and harassment behaviours only in the presence of the more threatening snake (Table 2.2: Hypothesis 1). Additionally, if Cape ground squirrels were capable of assessing and differentiating between the risk associated with two different high-risk venomous snakes (cobras and puff adders), then individuals would increase harassment and vigilant behaviours in the presence of actively hunting cobras when compared to the cryptic and sedentary puff adder (Table 2.2: Hypothesis 2).

Biology of the study animal

Female Cape ground squirrels live in matrilineal kin groups (called social groups, defined as a group of animals living together in the same burrow system) of one to three adult females, up to nine sub-adults of either sex, and up to four natal males (Waterman 1995; Scantlebury et al. 2008). Social groups live in burrow clusters (defined as an aggregation of burrow openings clearly separated from adjacent clusters by areas without burrows that are larger than the cluster area; Herzig-Straschil 1978). Normally only one social group inhabits a burrow cluster. Social groups are characterized by female philopatry and male-biased dispersal (Waterman 1995). Males may delay their dispersal

to stay in the social group for an extended period as natal males. In South Africa, up to 62.5% of males do not disperse by the age of 16 months, and natal males as old as four years of age have been documented (Scantlebury et al. 2008). Natal males provide alloparental care of related offspring and are presumed to gain indirect fitness benefits from delaying dispersal along with direct fitness benefits because they visit neighbouring social groups when a female is in oestrus (Waterman 1995; Scantlebury et al. 2008; Manjerovic 2010).

Female Cape ground squirrels are able to breed throughout the year and mate with multiple males during oestrus (Waterman 1998). Before parturition, females isolate themselves from the social group in a separate burrow outside the burrow cluster and remain isolated during lactation and return to the group after young emerge (Waterman 2002). Once the weaned young have joined the social group, interactions of mothers with offspring are indistinguishable from the interactions of other members of the social group with the offspring because of alloparental care (Waterman 1995). Litter loss in Cape ground squirrels is high, with 70% of all oestruses failing to produce successful litters (Waterman 1996; Pettitt et al. 2008). Juveniles in the first few months after emergence are most vulnerable to predation because they remain close to the burrow cluster even when the social group moves out to forage leaving them alone for long periods of time (J.M. Waterman pers. comm.). Female social groups are limited to three reproductive females by reproductive suppression and group fission; female social group size is most likely limited by increased juvenile mortality because as social groups get larger they must forage over greater distances, making juveniles more susceptible to predation (Waterman 2002).

Methods

Study Site

My study was conducted at S.A. Lombard Nature Reserve from June-October 2011. The reserve is located 17 km west of Bloemhof in the North West Province of South Africa (25°30'E, 27°35'S) and is approximately 3,660-ha of grassland consisting of Cymbopogon–Themeda veldt on a floodplain (Van Zyl 1965). The study site receives a mean annual rainfall of 502 mm (range 241–965 mm; years 1952–2004; Pettit & Waterman 2011). The mean maximum and minimum temperature between June-October 2011 was 24.2 °C, and 6.1 °C respectively. Reptiles I observed on the reserve during the study period included mole snakes (*Pseudaspis cana*), Cape cobras (*Naja nivea*), puff adders (*Bitis arietans*), and rock monitors (*Varanus albigularis*) (and see Unck et al. 2009 for additional predator data for this field site).

Trapping and Marking

I trapped Cape ground squirrels with Tomahawk live traps (15 x 15 x 50 cm; Tomahawk Live Trap Co., Tomahawk, WI, U.S.A.) baited with a mixture of peanut butter and chicken feed. I trapped and marked nine social groups consisting of 56 individuals between June and November 2011. Individuals were re-captured to maintain dye marks for identification at a distance as needed. I restrained squirrels in a cloth bag (Koprowski 2002) and each squirrel was sexed, aged, weighed, and checked for reproductive condition. Age classes are defined as follows; juveniles are less than six months of age, sub-adults are older than six months and have often reached adult body mass but are not reproductive, adults are reproductive individuals. Sexual maturity in

females was determined by observing if females had long swollen nipples. Nipples remain permanently swollen and are about 0.75 cm long after first breeding. All adult females in the study had reproduced and lactated in their lifetime and likely had older offspring in the social group (in other words natal males and sub-adults). Reproductive maturity in males was determined by observing if males were scrotal (Pettitt et al. 2008). Nipples were also used to estimate the mothers of juveniles as females wean offspring within the first 7 days after offspring emergence and the nipples become *temporarily* longer (1.5 cm) and stretched and flattened at this time (Waterman 1996; Pettitt et al. 2008). I was able to record nine individual females with flattened nipples that had emerged juveniles in their social group and these individuals are labelled as females with juveniles in my analyses.

I gave each squirrel a unique dye mark (Rodol D; Lowenstein and Sons, New York, NY, U.S.A.) for identification at a distance and a PIT tag (Avid Inc., Norco, CA, U.S.A. and Biomark, Boise, Idaho, U.S.A.) and freeze mark (Rood & Nellis 1980) for permanent individual identification. All research protocols were approved by the University of Manitoba's Animal Care and Use Committee (F10-030/1).

Predator Harassment trials

I investigated the possible benefits of predator harassment, as well as the abilities of the Cape ground squirrel to discriminate among different snake predators, by examining variation in harassment behaviour among individuals of different ages, sexes, and reproductive conditions with trials using three sympatric snake species. Snakes were presented to each social group in a wire cage (50 x 55 x 55 cm) constructed out of two

layers of wire mesh. The outer layer was chicken wire and the inner layer was nylon screen (Graw & Manser 2007). The squirrels could see, smell, and hear but could not touch the snake. I placed an empty wire mesh cage in the burrow cluster at least 48 hours prior to each trial, to allow the squirrels to habituate to it. I cleaned the cage with a 50% vinegar solution after each trial to remove olfactory cues left by the predator or the squirrels (though each species of snake was assigned its own cage to ensure no olfactory cues from the different snake species were mixed). I placed the snake in the burrow cluster within 5 m of where the social group was observed emerging the evening before. A trial began when the first squirrel emerged. I recorded squirrel behaviour with a camcorder (Canon Vixia HFM41 with 1.7X telephoto lens, Canon Inc. Tokyo, Japan.), and I used binoculars (10 X 50) and spotting scopes (20 X 60) (Bushnell Co., KS, U.S.A.) to monitor the squirrels' behaviours. I terminated the trials when all animals left the burrow cluster to forage (defined as 15 m away from the cage). I placed an empty cage in the burrow cluster as a control. For all trials, I randomized treatment order within burrow clusters as well as the order in which burrow clusters were tested using a random number generator and I waited at least 48 hours between trials at the same burrow cluster to reduce habituation to the stimuli. I used a Kestrel 3000 Pocket Weather Meter (Nielsen Kellerman, Chester, PA, U.S.A.) to record average and maximum wind speed (over a two-minute period) to ensure that mobbing trials were not performed during high wind periods (wind speed gusting to greater than 18 km/h), minimizing the effect of wind on behaviour (Fairbanks & Dobson 2007).

Snakes

Martin Smit and PG du Plessis from Pangaea Reptile Conservations Projects CC caught and cared for all the snakes used in my trials. Snakes were housed at the field site and provided with food and water until trials were finished. I used three sympatric snake species to represent three levels of risk to the Cape ground squirrel. The brown house snake (*Lamprophis capensis*) represents very low risk to the Cape ground squirrel. The brown house snake is a non-venomous constrictor that mainly hunts rodents and lizards (Akani et al. 2008). The individual I used was probably not large enough to consume an emerged juvenile squirrel and was not a threat to an adult-sized ground squirrel though no study on maximum prey size-snake size allometry has been conducted with this genus (King 2002). The puff adder represents an intermediate risk to Cape ground squirrels as it produces largely cytotoxic venom and is an ambush predator (Broadley 1990; Mallow et al. 2003). The snouted cobra (*Naja annulifera*) has mostly neurotoxic venom and is a diurnal active hunter (Broadley 1990; Joubert 1976). The snouted cobra represents the highest risk level to Cape ground squirrels because puff adders rely on cryptic ambush and would be less dangerous once discovered than an active hunting species (Graw & Manser 2007). Previous research found no support for proteolytic resistance in the sera of the Cape ground squirrel against either puff adder or snouted cobra venom suggesting envenomation from either species would be very costly for an individual Cape ground squirrel (Phillips et al. 2012)

To examine differences in the anti-predator response among individuals in the social group, I conducted trials with two wild-caught snouted cobras (TL = 168 and 147 cm). To examine predator discrimination in the Cape ground squirrel, I conducted trials

with two wild-caught puff adders (TL = 94 and 95 cm), two snouted cobras (as described above) and one brown house snake (TL = 77 cm). When more than one snake was available, I randomized which individual snake was used for each treatment by flipping a coin. To control habituation of the snakes I waited at least 48 hours between trials for each individual snake.

Data collection

I defined predator harassment as the approach of an individual to within 1 m of the snake (within the strike zone of the snakes used in the trials), which is accompanied by other behaviours, including piloerection of the tail, and flagging [a tail flag is the movement of the tail in a sweeping motion in front of the body (see Waterman & Roth 2007)]. I defined inspection as an approach of an individual to within 1 m of the snake with no harassment-associated behaviours; the squirrel's body must be oriented towards the snake. Inspection behaviour is assumed to impose lower risk and is presumably less energetically costly than mobbing behaviour because the individual is not actively harassing the snake (Shields 1984). I scored trial videos using JWatcher v1.0 (Blumstein et al. 2006) using an ethogram from Waterman (1995). For each individual in the nine social groups tested I recorded time spent ≤ 1 m from the snake (in the strike zone) in seconds as either 1) time spent harassing which I defined as the sum of all times an individual approaches the cage within 1 m and displays harassment behaviours (defined above) or 2) time spent inspecting which I defined as the sum of all times an individual approaches within 1 m and directs vigilant behaviour towards the cage. I recorded all snake-associated vigilance by recording vigilant behaviour within 5 m of the cage during the trial as a measure of perceived risk (Edwards & Waterman 2011). I scored mobbing

intensity by assigning a rank for each individual as follows: 0= no approaches within 1 m; 1= 1 inspection within 1 m; 2= multiple inspections within 1 m; 3= 1 mob with 5 or fewer tail flags; 4= 1 mob with 6 or more tail flags; 5= multiple mobs with 9 or fewer tail flags; 6= multiple mobs with 10 or more tail flags.

Statistical Analysis

Data were tested for normality using Shapiro-Wilk tests (Zar 1999). If data were normally distributed, I compared means among different age/sex classes and among the four treatments using General Linear Models (GLM) with sex/age class or treatment as a fixed factor using SPSS 11.5 for Windows (SPSS Inc., Chicago IL U.S.A.). If data were not normally distributed, even after transformations, I used non-parametric Wilcoxon signed-rank tests, Mann-Whitney U tests or Kruskal-Wallis tests and made multiple comparisons using Dunn's post-tests to control for experiment-wise error rate in GraphPad Prism 5.00 for Windows (San Diego, CA U.S.A.; Higgins 2004). I used sign tests to compare each age/sex classes' median mobbing durations with zero using SAS/STAT® Version [8] software (SAS institute Inc., Cary NC U.S.A.). I combined sub-adults of both sexes for data analysis as I did not detect differences between the sexes in duration of inspection, vigilance, or harassment behaviour and my sample sizes were small (Mann-Whitney U: mobbing, $U = 6$, $N_1 = 3$, $N_2 = 4$, $P = 1.0$; inspection, $U = 4.5$, $N_1 = 3$, $N_2 = 4$, $P = 0.59$; vigilance, $U = 2$, $N_1 = 3$, $N_2 = 4$, $P = 0.16$). For the analyses of vigilance and inspection behaviours among experimental treatments, I compared the arithmetic mean of each social group to avoid pseudoreplication. Means are reported with SE when parametric tests were used and reported with medians and interquartile ranges when nonparametric tests were conducted. Statistical significance was set at $P < 0.05$ for

all analyses except for Dunn's post-tests where significance was set at $P < 0.10$ because of small sample sizes and the conservativeness of Dunn's post-test decreases statistical power (Conover 1971). For non-significant results 95% confidence intervals (CI) are presented. Confidence intervals are considered more informative than power tests in determining the power of non-significant statistical tests as narrow intervals indicate a higher probability that the true effect size is near zero (Colegrave & Ruxton 2003).

Results

Individual mobbing participation

A total of 45 individuals were present during the snouted cobra trials (highest risk treatment) though only 17 individuals approached within 1 m and displayed any harassment behaviours (7 out of 9 females with juveniles, 3 out of 6 females without juveniles, 3 out of 8 natal males, 2 out of 7 sub-adults, and 2 out of 15 juveniles). Only mothers with juveniles had harassment durations that differed from zero (Sign tests: females with juveniles, 95% CI = 11.42, 107, $M = 3.5$, $N = 9$, $P = 0.02$; females without juveniles, 95% CI = -10.49, 42.29, $M = 1.5$, $N = 6$, $P = 0.25$; natal males, 95% CI = -0.62, 6.21, $M = 1.5$, $N = 8$, $P = 0.25$; sub-adults, 95% CI = -2.92, 10.37, $M = 1$, $N = 7$, $P = 0.5$). Juveniles rarely approached within 1 m of the snake and did not significantly contribute to harassing or inspecting the snouted cobra (Sign tests: harassing, 95% CI = -3.01, 13.83, $M = 1$, $N = 15$, $P = 0.5$; inspecting, $M = 1.5$, $N = 15$, $P = 0.25$).

When examining body mass, I found that sub-adults were significantly lighter than both natal males and adult females. Though there was no statistically significant difference between natal males and adult females (body mass mean \pm SE; adult females =

647.07 ± 10.72 g, $N = 15$, adult males = 653 ± 14.183 g, $N = 8$, sub-adults = 564.29 ± 15.162 g, $N = 7$, one-way ANOVA: $F_{2,28} = 11.96$ $p = 0.0002$).

When comparing among age/sex classes, I found significant differences in the amount of time individuals spent harassing the snouted cobra, with females with juveniles spending the most time harassing, and females without juveniles intermediate between mothers and natal males and sub-adults (Figure 2.1, Kruskal-Wallis test: $H_{4,30} = 9.03$, $P = 0.03$). Sex/age classes also differed significantly in harassment intensity, with mothers of juveniles displaying the highest intensity and non-mothers intermediate between mothers and the natal males and sub-adults (Figure 2.2, Kruskal-Wallis test: $H_{4,30} = 11.02$, $P = 0.01$).

Venomous and non-venomous snakes

Snake treatments differed significantly in the amount of time females with juveniles spent harassing, with the snouted cobra treatment having higher durations compared to both brown house snake and control trials (Figure 2.3, Kruskal-Wallis test: $H_{3,27} = 15.02$, $P = 0.005$). Treatments also differed significantly in mother harassment intensity with mothers harassing the snouted cobra most intensely (Figure 2.4, Kruskal-Wallis test: $H_{3,27} = 13.40$, $P = 0.0012$) and with significantly more tail flagging when compared to brown house snake and control treatments (Figure 2.5, Kruskal-Wallis test: $H_{3,27} = 15.00$, $P = 0.0006$).

Treatments differed significantly in the amount of time females with juveniles spent harassing the two venomous snakes (Figure 2.3, Kruskal-Wallis test: $H_{3,32} = 10.96$, $P = 0.0042$) and how intensely they mobbed (Figure 2.4, Kruskal-Wallis test: $H_{3,32} =$

11.33, $P = 0.0035$), with the snouted cobra having the highest durations and highest intensity. Treatments also differed significantly in the number of tail flags mothers displayed to the snouted cobras and puff adders but only responses to snouted cobras differed significantly from the control (Figure 2.5, Kruskal-Wallis test: $H_{3,32} = 10.32$, $P = 0.0057$).

Sex/age class variation in inspection and vigilant behaviour

I found no significant differences among sex/age classes and duration of inspection in seconds for any of the three snake treatments or control (Kruskal-Wallis tests median inspection durations \pm interquartile ranges are shown first: *control*, natal males = 0 ± 12.12 s, $N = 8$, females with juveniles = 0 ± 23.37 s, $N = 11$, females without juveniles = 0 ± 19.48 s, $N = 7$, sub-adults = 0 ± 15.8 s, $N = 6$, 95% CI = 3.22, 14.38, $H_{4,32} = 0.2$, $P = 0.98$; *brown house snake*, natal males = 8.34 ± 46.31 s, $N = 8$, females with juveniles = 11.52 ± 51.03 s $N = 6$, females without juveniles = 10.62 ± 16.88 s, $N = 6$, sub-adults = 0 ± 25.63 s, $N = 8$, 95% CI = 8.78, 53.38, $H_{4,28} = 1.25$, $P = 0.74$; *puff adder*, natal males = 0 ± 8.08 s, $N = 10$, females with juveniles = 0 ± 54.63 s, $N = 11$, females without juveniles = 79.96 ± 208.02 s, $N = 6$, sub-adults = 6.19 ± 126.05 s, $N = 5$, 95% CI = 20.29, 115.33, $H_{4,32} = 2.99$ $P = 0.39$; *snouted cobra*, natal males = 12.52 ± 52.11 s, $N = 8$, females with juveniles = 38.43 ± 47.89 s, $N = 10$, females without juveniles = 47.21 ± 294.35 s, $N = 5$, sub-adults = 80.96 ± 303.71 s, $N = 7$, 95% CI = 38.25, 132.83, $H_{4,30} = 1.96$, $P = 0.58$, 90%).

Similarly, when comparing the proportion of the trial spent vigilant, I found no significant differences among the sex/age classes in any of the treatments (GLM mean proportion vigilant \pm SE listed for all treatments: *control*, natal males = 15 ± 5 %, $N = 8$,

females with juveniles = 23 ± 4 %, $N = 11$, females without juveniles = 9 ± 5 %, $N = 7$, sub-adults = 14 ± 5 %, $N = 6$, 95% CI = 0.12, 0.20, $F_{4,41} = 1.7$, $P = 0.19$; *brown house snake*, natal males = 21 ± 7 %, $N = 8$, females with juveniles = 35 ± 9 %, $N = 6$, females without juveniles = 24 ± 8 %, $N = 6$, sub-adults = 26 ± 7 %, $N = 5$, 95% CI = 0.18, 0.31, $F_{4,33} = 0.54$, $P = 0.71$; *puff adder*, natal males = 31 ± 5 %, $N = 10$, females with juveniles = 40 ± 4 %, $N = 11$, females without juveniles = 26 ± 6 %, $N = 6$, sub-adults = 26 ± 7 %, $N = 5$, 95% CI = 0.29, 0.37, $F_{4,46} = 0.65$, $P = 0.63$; *snouted cobra*, natal males = 33 ± 8 %, $N = 8$, females with juveniles = 49 ± 7 %, $N = 10$, females without juveniles = 43 ± 10 %, $N = 5$, sub-adults = 38 ± 9 %, $N = 7$, 95% CI = 0.18, 0.65, $F_{4,44} = 0.9$, $P = 0.6$).

Inspection and vigilance between venomous and non-venomous snakes

I found significant differences among treatments when I compared group mean inspection duration. The inspection times were significantly longer during snouted cobra trials (Figure 2.6, Kruskal-Wallis test: $H_{4,36} = 10.76$, $P = 0.01$). I also found significant differences among treatments when I compared group means of the proportion of a trial spent vigilant. The proportion of vigilance in snouted cobra trials was significantly higher than the control trials and puff adder and brown house snake trials were intermediate between snouted cobra and control trials (Figure 2.7, GLM: $F_{3,8} = 4.43$, $P = 0.01$).

Discussion

Cape ground squirrel predator harassment behaviour was very similar to other described sciurids (Owings & Coss 1977; Loughry 1987; Owings et al. 2001). Cape ground squirrels approached snakes and investigated them at close range by rising up onto their back legs and orienting themselves towards the snake. Some individuals waved

their piloerected tails from side to side (flag) and also held their tails lateral to their bodies similar to tail sweeping documented in rock squirrels (*Otospermophilus variegatus*) (Owings et al. 2001). Cape ground squirrels never vocalized and never threw substrate during the trials unlike both rock squirrels and California ground squirrels (Owings & Coss 1977; Owings et al. 2001).

Cape ground squirrels approached all three species of snake but they adjusted their response to the risk level the snake potentially posed. The snouted cobra trials had the highest vigilance and inspection behaviours from the squirrels, suggesting they saw this species as the highest risk. The snouted cobra also elicited the strongest predator harassment response with the longest durations and highest intensity of all treatments. Adult females with emerged juvenile offspring took the most risks in the social group by harassing the snouted cobra for longer durations and with more intensity than any other group members. Adult females without juveniles were intermediate between females with juveniles and all other sex/age classes in both harassment duration and intensity and natal males, despite being reproductively mature, behaved very similarly to sub-adult individuals during the harassment trials. Contrary to the patterns seen in predator harassment, sex/age classes behaved very similarly when inspecting all snake treatments and were similarly vigilant during the trials.

It appears that Cape ground squirrels actively harass the high risk snake species, suggesting that predator harassment is primarily anti-predator in nature. This finding supports previous observations of this species only harassing cobras, puff adders, and monitor lizards (Waterman 1997; Waterman & Roth 2007). Suricates, a mongoose species of similar body size to the Cape ground squirrel, harassed snakes along with a

variety of other species and displayed similar discrimination abilities among snake species; with cobras mobbed for longer durations than puff adders or non-venomous snakes (Graw and Manser 2007). Another species of mongoose, the dwarf mongoose, also harasses snakes including puff adders, spitting cobras (*Naja nigricollis*) and black mambas (*Dendroaspis polylepis*). There was no size or species discrimination noted in the dwarf mongoose but their harassment could be multi-functional as they are also known to prey upon smaller snakes (Rasa 1987).

The dynamics observed between passive (inspection) and active (harassment) defence in groups has been documented in many other species. In barn swallows, breeding pairs are more likely to actively harass predators than juveniles or non-breeding pairs (Shields 1984). In Formosan squirrels (*Callosciurus erythraeus*) and California ground squirrels, females with offspring are more likely to actively mob while males and sub-adults are more likely to passively inspect (Tamura 1989; Swaisgood et al. 1999). Both species have a promiscuous mating system conferring low certainty of paternity (Tamura et al. 1988; Boellstorff et al. 1994). As predicted with this mating system, males provide no paternal care of offspring and active snake-directed anti-predator behaviour is also maternally biased as mothers spend significantly more time and effort in anti-snake activity (Tamura 1989; Swaisgood et al. 1999). In the black-tailed prairie dog (*Cynomys ludovicianus*), a cooperative breeder, non-breeding helpers provide some offspring care and mobbing behaviour seems to be, in part, protection of offspring. However, males participate the most in snake harassment (Loughry 1987). A male bias in snake-directed behaviour occurs because females are unlikely to mate multiply and the males are thus most likely to be the fathers of the offspring (Hoogland and Foltz 1982). As a result,

males invest more in protecting offspring directly (Hoogland and Foltz 1982; Loughry 1987).

Predator harassment in Cape ground squirrels most resembles that of sciurids that are not cooperative breeders, like the California ground squirrel and Formosan ground squirrel (Tamura 1989; Swaisgood et al. 1999). Natal males, females without juveniles, and sub-adults' harassment of the snouted cobra did not differ statistically from zero, which does not support the hypothesis that snake-predator harassment is a product of alloparental care. However, all group members equally contributed to vigilance and inspection of the snake during the trials. Although vigilance and inspection behaviours are probably multi-functional for the individual, they may also represent an expression of alloparental care and cooperation as a means to alert other group members to a threat and to increase vigilance, similar to cooperatively breeding suricates (Graw & Manser 2007).

Several cooperatively breeding small carnivores that share similar habitat, body size and snake predators to Cape ground squirrels engage in group snake harassment. Alloparenting has been documented in the suricate and alloparents participate in group predator harassment though mobbing intensity increases with age and is biased towards males in the social group (Graw & Manser 2007). In dwarf mongooses subordinate adults and sub-adult individuals (the alloparents of the social group) lead most attacks on predators while the dominant male and female usually remain a few meters away during the mobbing bout (Rasa 1987).

Predator harassment can be multi-functional and there are other hypotheses to explain high mobbing rates and intensity by females with juveniles other than maternal

care. Active harassment could be “selfish” by increasing the chances of survival to the individual (Dugatkin and Godin 1992). If predator harassment was for selfish reasons, I would expect to see similar harassment behaviour among age/sex classes. Even though that was not the case during my trials, variations in the mobbing-related risk level to each individual, due to size/experience could explain the patterns. Adult females and natal males are significantly larger than sub-adult individuals. However, natal males displayed little difference in behaviour from the smaller sub-adults, suggesting it is unlikely that differing levels of risk of envenomation due to body mass could explain the observed pattern in harassment behaviours. I was not able to separate the effect of age/experience and reproductive condition as I did not know if females with juveniles were older than females without juveniles and natal males in my trials. However, as some males do not disperse until they are 3-4 years old and females can have their first oestrus between 7-9 months of age (Waterman 2002; Petitt et al. 2008; Scantlebury et al. 2008), it is unlikely there were age differences. Natal males behaved similarly to sub-adult individuals, and snake detection abilities and associated anti-snake behaviours have been documented to develop very early in other sciurids (Owings & Coss 1977). If experience plays a role in harassment behaviours, I would expect to see similar levels of harassment in natal males and females without juveniles and lower levels in younger sub-adults. As this is not the case, I do not think the low participation of natal males and sub-adults can be explained by lack of experience alone. Self-defence may play a role in the decision to mob in the Cape ground squirrel but because females with juveniles participate the most in snake-predator harassment I conclude one major function of predator harassment in this species is maternal care.

Adult females could also be receiving benefits for participating through mutualism, or reciprocal altruism among females in a social group or through indirect and direct fitness benefits accrued from helping older offspring and closely-related kin as females without juvenile offspring were intermediate between females with juveniles and all other age/sex classes in harassment duration and intensity. This is explained by the likelihood that all adult females have older offspring in the social group. Older offspring are less vulnerable to predation by cobras, which would explain females without juvenile's lower participation than females with juvenile offspring. Juvenile individuals are at most risk for cobra predation because they are left alone at the burrow cluster while the social group moves out to forage during the day (Waterman 2002; J.M. Waterman pers. comm.). Further research would need to be done in order to understand all the benefits involved in predator harassment in adult female Cape ground squirrels.

In conclusion, predator harassment appears to be an anti-predator behaviour used against snakes in the Cape ground squirrel. Mobbing behaviour is maternally biased and most likely serves to protect more vulnerable juvenile offspring and raise vigilance in the social group. Cape ground squirrels are able to discriminate among different sympatric snake species and grade their response to the level of risk those snakes impose. Although Cape ground squirrels are facultative cooperative breeders, mobbing behaviour does not appear to be a function of alloparental care.

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Tables and figures

Table 2.1: Who in the social group is participating in predator harassment? Hypotheses, predictions and results for Cape ground squirrels.

Hypothesis 1: Snake-predator harassment is part of alloparental care		Support
Predictions		
1	Individuals of all reproductive classes participate in harassing snakes in the burrow cluster	No
Hypothesis 2: Snake-predator harassment is maternal behaviour		
Predictions		
1	Only mothers with juveniles exhibit predator harassment behaviours	Yes
2	Mothers risk most (higher frequencies, longer durations, and higher intensities)	Yes

Table 2.2: Can they discriminate among snake species? Hypotheses and predictions for Cape ground squirrels.

Hypothesis 1: Cape ground squirrels discriminate between snakes that represent no risk and snakes that represent higher risk		Support
Predictions		
1	Individuals increase vigilance in the presence of venomous snakes	Yes
2	Individuals increase harassment behaviours in the presence of venomous snakes	Yes
Hypotheses 2: Cape ground squirrels are capable of assessing the risk between two high risk venomous snakes, treating the venomous snake that is more mobile as more threatening		
Predictions		
1	Individuals increase vigilance towards more actively hunting cobras	No
2	Mobbing individuals increase harassment behaviours directed at cobras	Yes

Figure 2.1: Comparing predator harassment duration (s) during snouted cobra trials among age/sex classes of Cape ground squirrels. Median values with the interquartile range are shown for each of the four age/sex classes: females with juveniles, females without juveniles, natal males, and sub-adults. Comparisons for all pairs using Dunn's post-tests, and sample sizes shown above each treatment.

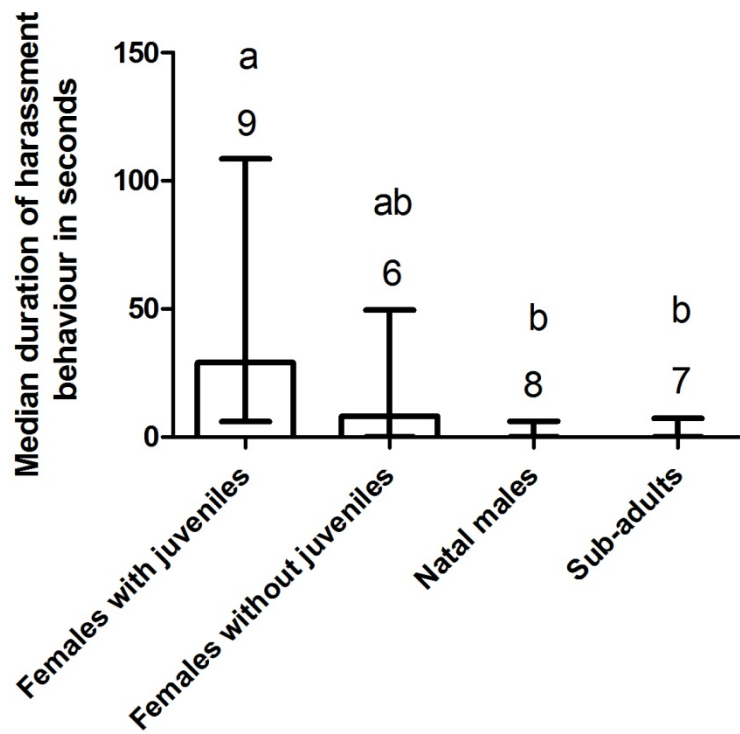


Figure 2.2: Comparing median mobbing intensity among age/sex classes of Cape ground squirrels during snouted cobra trials. Median values with the interquartile range are shown for each of the four age/sex classes: females with juveniles, females without juveniles, natal males, and sub-adults. Comparisons for all pairs using Dunn's post-tests, and sample sizes shown above each treatment.

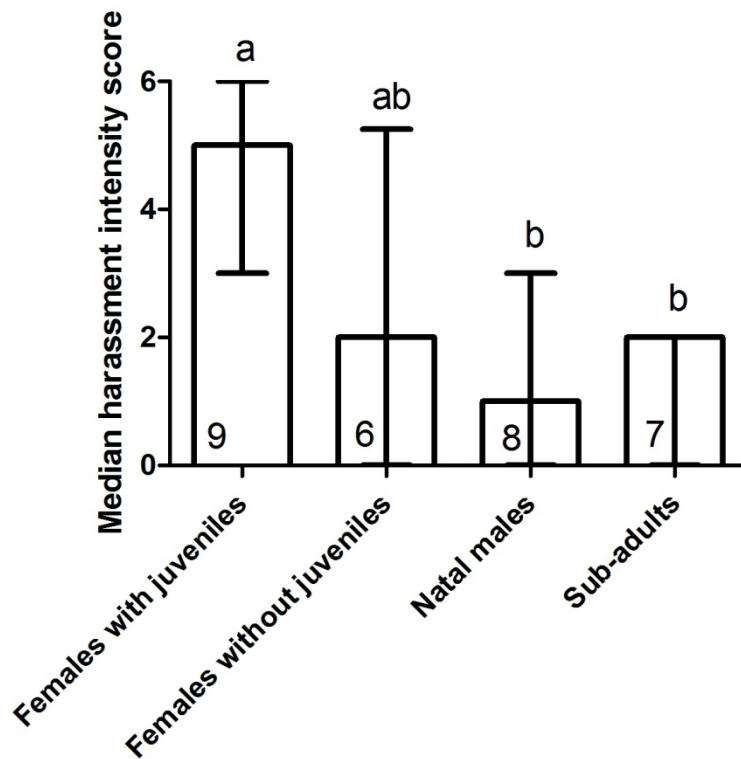


Figure 2.3: Comparing median predator harassment duration in seconds of Cape ground squirrels when exposed to different snake species. Median values with the interquartile range are shown for each of the four treatments: control, brown house snake, puff adder and snouted cobra. Comparisons for all pairs using Dunn's post-tests, and sample sizes shown above each treatment.

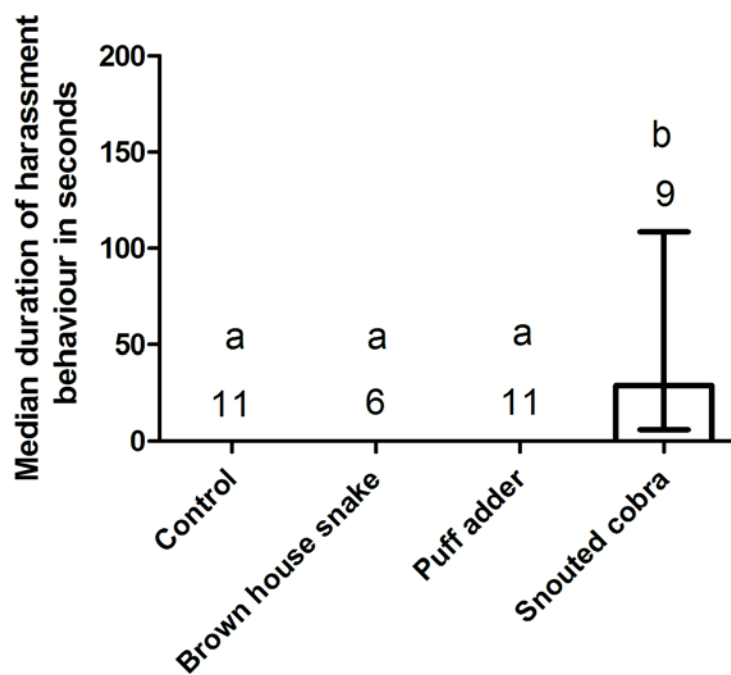


Figure 2.4: Comparing median predator harassment intensity of Cape ground squirrels exposed to different snake species. Median values with the interquartile range are shown for each of the four treatments: control, brown house snake, puff adder and snouted cobra. Comparisons for all pairs using Dunn's post-tests, and sample sizes shown above each treatment.

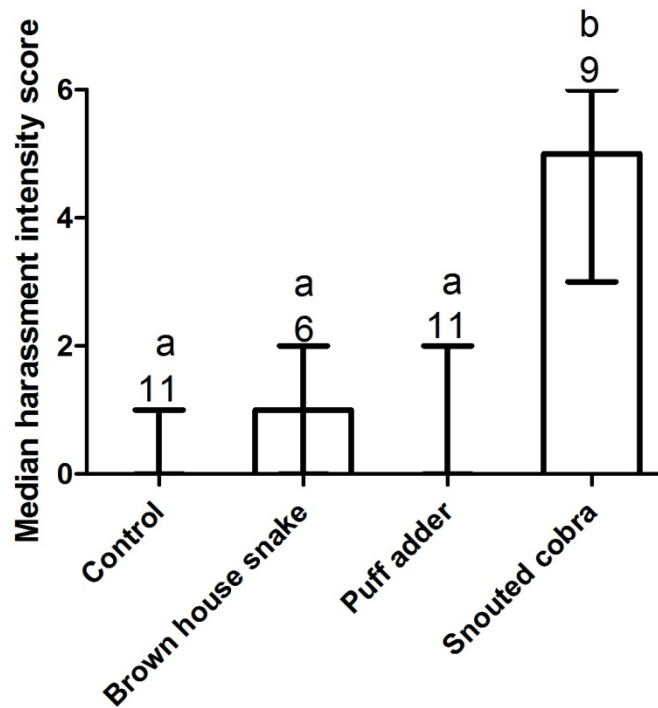


Figure 2.5: Comparing median tail flagging frequency of Cape ground squirrels exposed to different snakes species. Median values with the interquartile range are shown for each of the four treatments: control, brown house snake, puff adder and snouted cobra. Comparisons for all pairs using Dunn's post-tests, and sample sizes shown above each treatment.

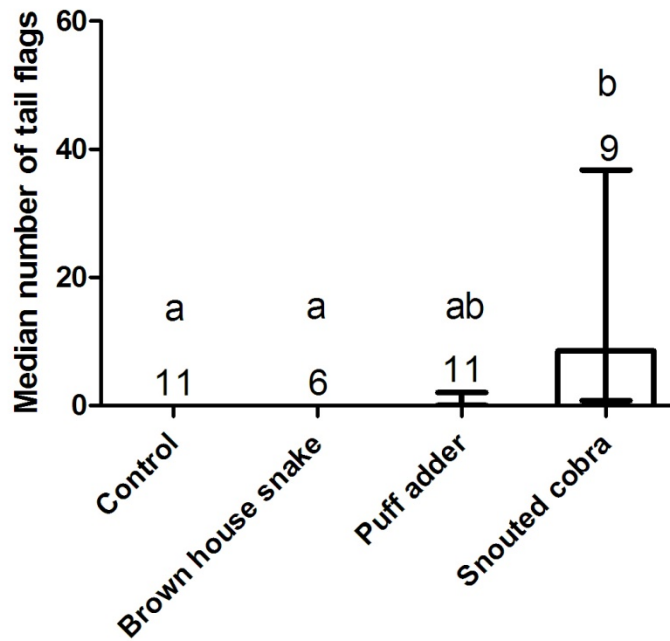


Figure 2.6: Median inspection duration in seconds of Cape ground squirrel social groups (means) exposed to different snake species. Median values with the interquartile range are shown for each of the four treatments: control, brown house snake, puff adder and snouted cobra. Comparisons for all pairs using Dunn's post-tests, and sample sizes shown above each treatment.

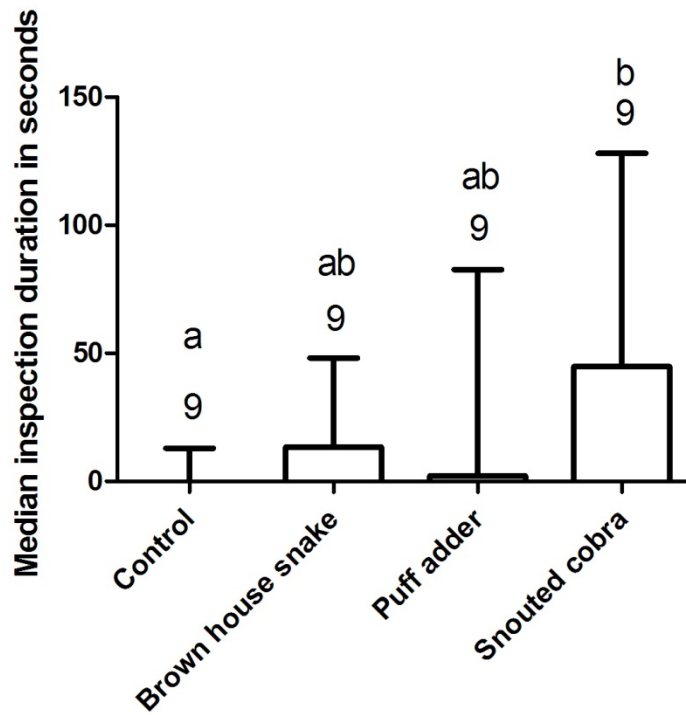
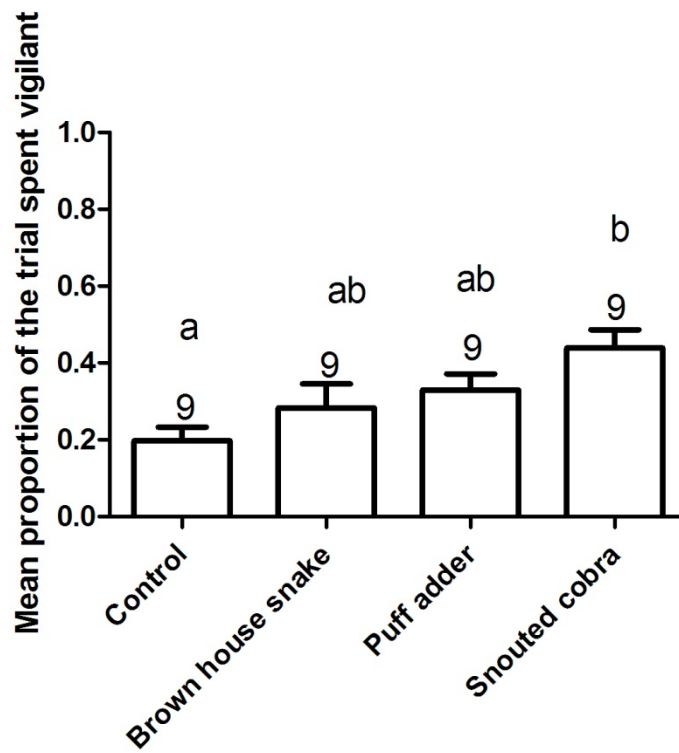


Figure 2.7: Cape ground squirrel social group mean proportion of the trial spent vigilant among treatments (arcsine transformation). Mean values (\pm SE) are shown for each of the four treatments: control, brown house snake, puff adder and snouted cobra.

Comparisons for all pairs using Tukey-Kramer HSD, and sample sizes shown above each treatment.



Chapter III: Olfactory snake-predator discrimination in the Cape ground squirrel

Abstract

Many small mammals have the ability to discriminate between predators by odour, particularly species that are nocturnal and solitary and where visual cues would not be as reliable. The Cape ground squirrel (*Xerus inauris*) is a semi-fossorial, diurnal mammal from southern Africa. Cape ground squirrels encounter multiple species of predatory snake that pursue individuals underground where visual and social cues are limited. I assessed whether Cape ground squirrels use odours to discriminate among snakes by presenting a non-venomous snake, a venomous snake and a control odour collected on polyethylene cubes to 11 adult female squirrels. Cape ground squirrels responded by inspecting the cube, displaying snake harassment-associated behaviours and decreasing time spent in close proximity to snake odours when compared with a control. They also displayed discrimination between two snake species by increasing the frequency of cube inspection with venomous snake odours when compared to non-venomous snake odours. I conclude that Cape ground squirrels respond with snake specific anti-predator behaviours when presented olfactory cues alone. Olfactory discrimination may be maintained by the decreased utility of other methods of predator detection; sight and group detection, in below-ground encounters.

Introduction

Prey species use a variety of senses to recognize predators (Apfelbach et al. 2005). Visual cues are especially important for predator detection by species living in open habitats (Mitchell & Skinner 2003). Auditory signals, especially alarm calls

produced by other potential prey individuals, are also important for social species that cannot always rely on vision (Warkentin et al. 2001). Olfactory cues (particularly predator odour) are another major signal, especially in prey that are nocturnal or otherwise visually limited (like many fossorial mammals) (Apfelbach et al. 2005).

Many species are capable of identifying predators by their odour, including several species of ungulates and rodents (reviewed in Apfelbach et al. 2005). The majority of research examining responses to predator chemical cues has focused on how prey alter their activity patterns (Borowski 1998; Borowski & Owadowska 2001) and shift habitat (Dickman 1992; Ward & Macdonald 1997) in the presence of predator odours. Some prey species are able to discriminate between specific predator odour cues and modify their behaviour to match the perceived risk (Blumstein et al. 2008). The mountain log skink (*Pseudemoia entrecasteauxii*) discriminates among snake predators, avoiding refuges tainted with snake odours from their most common snake predator and choosing refuges tainted with the odour of less common predatory snakes (Stapley 2003). A freshwater snail (*Physella heterostropha pomila*) also exhibits increased avoidance behaviours as predator risk increases (McCarthy & Fisher 2000).

The Cape ground squirrel (*Xerus inauris*) is a highly social, diurnal, semi-fossorial sciurid from southern Africa (Waterman 1995; Skurski & Waterman 2005). Cape ground squirrels live in open habitats and use both visual and auditory cues to detect predators (Furrer & Manser 2009; Unck et al. 2009; Edwards & Waterman 2011). Cape ground squirrels alter their behaviour as predation risk changes, as they are less vigilant in areas with lower predator densities (Unck et al. 2009). When Cape ground squirrels encounter snakes above ground they approach and closely inspect the snake.

Some individuals also actively harass the snake (described as approaching within striking distance and flagging their piloerected tails) (Chapter 2; Waterman 1997; Waterman & Roth 2007). Cape ground squirrels display predator discrimination abilities among sympatric snake species, adjusting their behaviours in response to different snake predators (Chapter 2). Several species of snake predators use squirrel burrows for hunting or resting (Alexander & Marais 2007). Underground encounters with snakes would most likely reduce the effectiveness of using visual and auditory cues (alarm calls) whereas chemical cues (odours) would be effective in identifying the presence of snake-predators prior to and during emergence (Towers & Coss 1990).

In this study, I tested whether olfaction is used in snake-predator discrimination by Cape ground squirrels. The objectives of my study were to: A) determine if Cape ground squirrels responded to the odour of sympatric-snake predators; and, B) determine if Cape ground squirrels were able to discriminate among snake-predator species by chemical cues alone. I tested the hypothesis that if Cape ground squirrels responded to odour cues from snakes then snake odours would elicit some harassment-associated behaviour including tail piloerection and tail flagging. I also predicted that snake odours would elicit close inspection of the scent source. Inspection is defined as approaching and orienting the nose within 25 cm of the scent source. Both inspection and harassment behaviours are observed when the species encounters live snakes (see Chapter 2). Additionally, I predict that individuals would decrease the proportion of time spent in close proximity to the snake chemical cue compared with a neutral control. I also examined the hypothesis that if Cape ground squirrels used olfactory cues to discriminate *among* snake predators, they would show more harassment and inspection behaviours

towards venomous snake odour cues when compared with a non-venomous snake odour cue. I also predicted that individuals would spend less time in close proximity to the venomous snake odour cues when compared with the non-venomous odour cue.

Methods

Study Site

My study was conducted at S.A. Lombard Nature Reserve from June-August 2011. The reserve is located 17 km west of Bloemhof in the North West Province of South Africa (25°30'E, 27°35'S) and is approximately 3,660-ha of Kalahari grassland consisting of *Cymbopogon*–*Themeda* veldt on a floodplain (Van Zyl 1965). The study site receives a mean annual rainfall of 502 mm (range 241–965 mm; years 1952–2004; Pettit & Waterman 2011). The mean maximum and minimum temperatures between June-August 2011 were 15.6 °C, and 0.4 °C respectively.

Trapping and Marking

I trapped 11 Cape ground squirrel social groups (61 individuals) with Tomahawk live traps (15 x 15 x 50cm; Tomahawk Live Trap Co., Tomahawk, WI, U.S.A.) baited with a mixture of peanut butter and chicken feed. Individuals were re-captured to maintain dye marks for identification at a distance as needed. I restrained squirrels in a cloth bag (Koprowski 2002) and each squirrel was sexed, aged, weighed, and checked for reproductive condition. Only adult females were used for the olfactory discrimination trials to avoid age/sex class behavioural variation. Adult females were sexually mature individuals, indicated by long swollen nipples (nipples remain permanently swollen after first breeding; Waterman 1996; Pettitt et al. 2008;). I gave each squirrel a unique dye

mark (Rodol D; Lowenstein and Sons, New York, NY, U.S.A.) for identification at a distance and a PIT tag (Avid Inc., Norco, CA, U.S.A. and Biomark, Boise, Idaho, U.S.A.) and freeze mark (Rood & Nellis 1980) for permanent individual identification. All research protocols were approved by the University of Manitoba's Animal Care and Use Committee (F10-030/1 and F11-038).

Olfactory discrimination trials

I tested olfactory snake-predator discrimination in the Cape ground squirrel by using snake sheds from two sympatric snake predators; the snouted cobra (*Naja annulifera*) and the mole snake (*Pseudopsis cana*). The snouted cobra has mostly neurotoxic venom and is a diurnal active hunter (Broadley 1990; Joubert 1976). The mole snake is a non-venomous constrictor (Alexander & Marais 2007). Snouted cobras represent a higher risk to squirrels compared to mole snakes because of their potent venom (Graw & Manser 2007). Cape ground squirrels are not resistant to the venom of snouted cobras (Phillips et al. 2012) suggesting a high cost of envenomation. Both cobras and mole snakes were documented at the field site during the 2011 field season (see Chapter 2). I collected three sheds from each snake species opportunistically in the field and individually bagged and stored them in a -20⁰C freezer until the experiments were performed. I randomly chose among the three sheds for each trial by assigning each shed a number and randomly choosing a number out of a bag.

I presented the snake-shed odour, but not the sheds themselves, to remove any reaction to the visual stimulus of the snake shed. I used polyethylene blocks (¾ x ¾ x ¾ cm, Acryl Design Ltd. Winnipeg, MB) to collect the odours from the sheds. Blocks were

first soaked for five minutes in a 50% vinegar solution, rinsed with water and then placed in the sun to air dry. To collect snake scent, I placed a block in the bag with a shed 30 minutes prior to a trial. I used a washed block placed in an empty plastic bag as a control. I presented each scent treatment (control, non-venomous and venomous) one at a time to 11 social groups by placing the cube at a burrow entrance on a 15 x 50 cm sheet of polypropylene tarp (tarp was washed in similar way to the cubes) when squirrels were underground. I placed a tarp with an experimental block within 5m of a burrow where an adult female in the social group had immersed. To ensure that animals approached the tarp, I also placed 1 tablespoon of trapping bait on the opposite side of the tarp from the cube. The trial began when the target female emerged from the burrow. I observed the squirrels with 10-45 X 50 binoculars and a spotting scope (20 X 60) (Bushnell Co., KS, U.S.A.) and recorded all behaviours (focal animal sampling, Altmann 1974) for 15 minutes on an 8 MB iPod Touch (Apple Inc. California U.S.A.). I randomized treatment order within burrow clusters as well as the order in which burrow clusters were tested using a random number generator and waited at least 48 hours between trials at the same burrow cluster to reduce habituation of squirrels to the experimental procedure. I used a Kestrel 3000 Pocket Weather Meter (Nielsen Kellerman, Chester, PA, U.S.A.) to record mean and maximum wind speed (over a two-minute period) to ensure that the trials were not performed during high wind periods (wind speed gusting to greater than 18 km/h), minimizing the effect of wind on squirrel behaviour and scent dispersal.

Data Analysis

I scored audio recordings of the focal animal sampling with JWatcher v1.0 (Blumstein et al. 2006) using an ethogram from Waterman (1995). For each focal individual I recorded: a) number of harassment-associated behaviours observed within 1 m of the cube. Harassment-associated behaviours included: piloerection of the tail, tail flagging (movement of the tail in a sweeping motion in front of the body) and tail sweeping (a piloerected tail held lateral to the body; see Chapter 2; Waterman & Roth 2007 for more detailed descriptions of harassment behaviours). b) the number of times an individual inspected the scent cube (defined as approaching the cube within 0.5 m while orienting its nose within 25 cm of the cube); and, c) time (s) spent ≤ 0.5 m from the cube as a measure of time spent in close proximity to the odour cue.

I tested data for normality using Shapiro-Wilk tests (Zar 1999). If data were not normally distributed, I transformed the data (arcsine) and compared behaviours among the 3 treatments (venomous, non-venomous, control) using a two-way ANOVA with social group as a factor in JMP Version 8 as the experiment was a randomized complete block design with each social group receiving all three treatments (SAS Institute Inc., Cary, NC U.S.A). If I was unable to normalized the data, I compared treatments using Friedman's tests blocking for social group and made multiple comparisons using Dunn's post-tests to control for experiment-wise error rate in GraphPad Prism 5.00 for Windows (San Diego, CA U.S.A; Higgins 2004). Data are reported as means with standard error (SE) when parametric tests were used and as medians and interquartile ranges when nonparametric tests were used. Statistical significance was set at $P < 0.05$.

Results

Individuals spent significantly less time near the venomous scent treatment (within 0.5 m) while non-venomous treatments were intermediate between the venomous and control treatments (Figure 3.1, two-way ANOVA: $F_{2,32} = 5.92$, $p = 0.01$). Individuals also investigated the venomous scent cube significantly more often than both the non-venomous and control treatments (Figure 3.2, Friedman test: $\chi^2_2 = 12.5$, $p = 0.0006$). There were also differences in the frequency of harassment-associated behaviours. Venomous treatments elicited significantly more harassment behaviours than the control while non-venomous treatment's harassment behaviour frequency was intermediate between control and venomous treatments (Figure 3.3, Friedman test: $\chi^2_2 = 9.5$, $p = 0.004$).

Discussion

Cape ground squirrels respond to snake-predator odours without visual or auditory cues by inspecting the odour source and displaying snake harassment-associated behaviours. Cape ground squirrels also respond to snake odours by decreasing the time they spend near the odour source. They can discriminate between two common snake species and treat the highly venomous snouted cobra's scent differently by increasing the frequency with which they inspect the cobra odour when compared to the non-venomous mole snake odour.

It has been suggested that small mammals that are both social and diurnal like Cape ground squirrels have reduced their reliance on olfactory cues to detect predators as they can use visual cues, vigilance of other individuals and alarms calls from both

conspecifics and heterospecifics to detect predators (Clutton-Brock et al. 1999; Belton et al. 2007; Waterman & Roth 2007; Edwards & Waterman 2010). However, snake predators like the very common puff adder (*Bitis arietans*), often use crypsis to ambush prey, making detection using visual cues and vigilance more difficult. Additionally, many snake predator species share burrows with Cape ground squirrels increasing the likelihood that a squirrel would encounter a snake while underground where visibility and social cues would be limited (Alexander & Marais 2007; Towers & Coss 1990). As Cape ground squirrels also move away from their burrows during the day to forage, the ability to detect a snake underground when returning to a burrow could be especially important to their survival (Waterman 2002). All of these factors would influence the Cape ground squirrels reliance on olfactory cues to identify snake-predators.

Other rodent species in the family Sciuridae display olfactory discrimination abilities of potential predators. The response of California ground squirrels (*Otospermophilus beecheyi*) to both venomous and non-venomous snakes in a simulated burrow system where no visual cues were available to the squirrels included sand kicking (a snake-predator specific behaviour in this species) and cautious investigation demonstrating that they used either olfactory or auditory cues to identify snakes underground (Coss & Owings 1978; Towers & Coss 1990). Individual California ground squirrels also approached snakes placed in clear perforated bags more often and had higher rates of sand kicking when compared to trials in which the snakes were presented in clear sealed bags (Hennessy & Owings 1978). Yellow-bellied marmots (*Marmota flaviventris*), another social scuirid, also displayed discrimination abilities between predators and non-predators by increasing their rate of investigation of cotton balls

soaked in predator urine when compared to herbivore urine (Blumstein et al. 2008). When snake-predators were presented to Siberian chipmunks (*Eutamias sibiricus*) using only olfactory cues, the chipmunks reacted similarly to Cape ground squirrels by inspecting and tail-shaking towards the snake treatments more frequently than any other treatment (tortoise, frog, eel, quail) (Kobayashi 1987). Also woodchucks (*Marmota monax*) and gray squirrels (*Sciurus carolinensis*) were found to avoid foraging near the urine of potential predators (Bean et al. 1995; Rosell 2001). Even though visual and social cues are highly important for social and diurnal species it appears that olfaction still plays an important role in discrimination especially of predators that are more difficult to detect visually for many rodent species.

Many studies have examined predator avoidance by comparing trapping rate between clean live-traps and traps contaminated with predator odours and have found many prey species avoid contaminated traps (Stoddart 1976; Gorman 1984; Robinson 1990; Tobin et al. 1997). Cape ground squirrels have been found to avoid traps contaminated with predator faeces, in addition to increasing their vigilance near the contaminated traps, suggesting they can identify mammalian predators by odour (Belton et al. 2007). Together with this study there is evidence that Cape ground squirrels respond to multiple predatory odours of their common terrestrial predators.

Cape ground squirrels are able to use olfactory cues to identify snakes without any other secondary visual or auditory cue from either the predator or other ground squirrels. Using olfaction to detect snake-predators would prove advantageous when squirrels cannot use their primary methods of predator detection; sight and group detection when they enter a burrow (Belton et al 2007; Edwards & Waterman 2010).

Further research could examine other sensory cues important in snake-predator discrimination such as differences in snake responses to Cape ground squirrel harassment.

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Figures

Figure 3.1: Comparing mean time (\pm SE) spent within 0.5 m of the mat during the trial by Cape ground squirrels for each of the three treatments: control, non-venomous and venomous snake odour. Comparisons for all pairs using Tukey-Kramer HSD and sample sizes are shown above each treatment. Raw data are shown but data were transformed using arcsine for analysis.

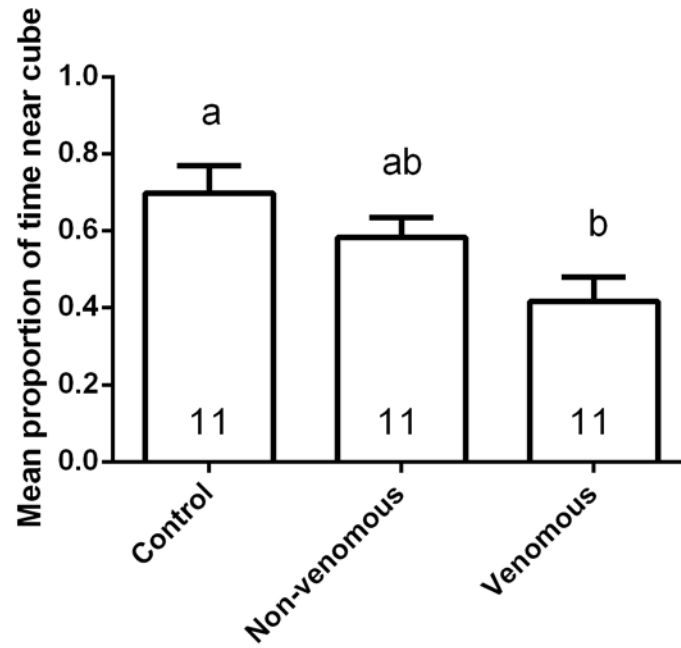


Figure 3.2: Comparing cube inspection frequency by Cape ground squirrels and snake scent treatment (control, non-venomous and venomous snake odours). Median values with the interquartile range are shown for each of the three treatments. Comparisons for all pairs using Dunn's post-tests, and sample sizes are shown above each treatment.

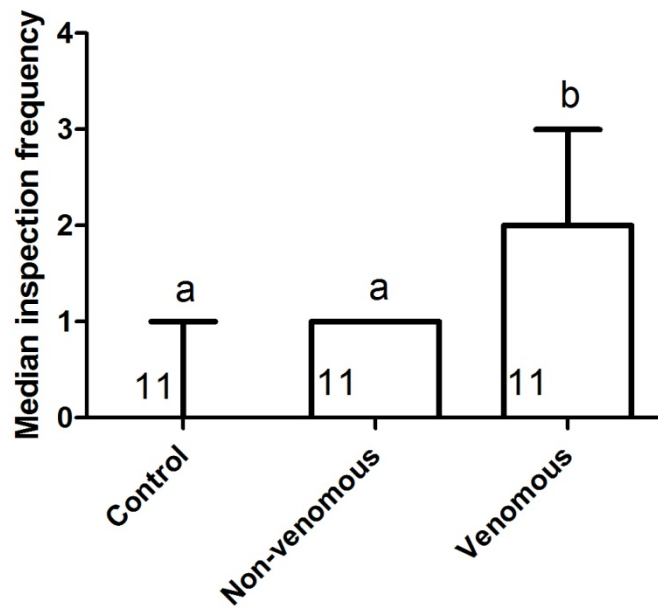
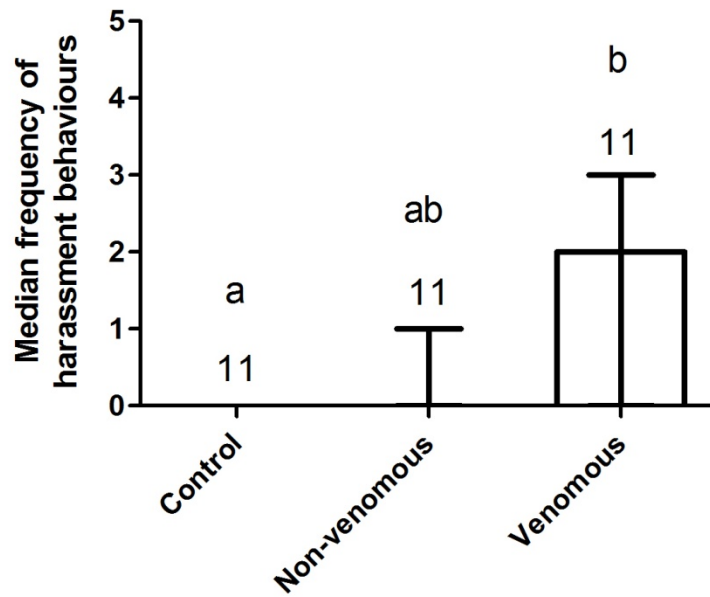


Figure 3.3: Comparing harassment associated behaviour frequency in Cape ground squirrels with different scents (control, non-venomous and venomous). Median values with the interquartile range are shown for each of the three treatments. Comparisons for all pairs using Dunn's post-tests, and sample sizes are shown above each treatment.



Chapter IV: No evidence for proteolytic venom resistance in southern African ground squirrels

Abstract

Many mammalian species that interact with venomous snakes show resistances to venoms. The family Sciuridae has several North American members that harass venomous snakes and show proteolytic resistances in their sera. I examined sera collected from an African ground squirrel (*Xerus inauris*) against two sympatric venomous snakes (*Bitis arietans* and *Naja annulifera*) and found no support for proteolytic resistance. Our results add to our understanding of the risks in predator defence within the family Sciuridae in general and to the trade-offs faced by Cape ground squirrels in particular.

Introduction

Animal venoms are a complex mixture of proteins and peptides that induce many destructive physiological effects for a variety of purposes, including prey capture (Fry et al. 2008; Jansa & Voss 2011), digestion (Thomas & Pough 1979) and defence (Kardong 1982). Some animals that interact with venomous snakes have physiological resistance to venom. As a predator, the Indian gray mongoose (*Herpestes edwardsii*) is resistant to the haemorrhagic effects caused by the venom of many snake species (Tomihara et al. 1990). California ground squirrels [*Otospermophilus beecheyi* formerly *Spermophilus* (Helgen et al. 2009)] defend against snake predation by active harassment and have resistance against the proteolytic activity of the venom from northern Pacific rattlesnakes (*Crotalus oreganus*) (Poran et al. 1987; Biardi 2000).

The Cape ground squirrel (*Xerus inauris*) is a ground-dwelling sciurid that inhabits the arid regions of southern Africa (Skurski & Waterman 2005). The Cape

ground squirrel, similar to the California ground squirrel, approaches and harasses several venomous species of snake and snake harassment in the Cape ground squirrel is the focus of ongoing research (Owings & Coss 1977; Waterman 1997; Waterman & Roth 2007). No data are available on the Cape ground squirrel surviving envenomation nor is it known if the Cape ground squirrel has similar proteolytic resistance to native venomous snakes.

I focused on two species of venomous snakes that prey on rodents and live sympatrically with the Cape ground squirrel, the puff adder (*Bitis arietans*) and the snouted cobra (*Naja annulifera*) (Phelps 1989; Broadley 1990; Shine et al. 2007). The venom of puff adders causes severe local and systemic effects to tissue including necrosis (Warrell et al. 1975; Rippey et al. 1976; Mallow et al. 2003). The venom composition of the snouted cobra is similar to other members of *Naja*, having both neurological and cytotoxic properties (Joubert 1976). No data are currently available for mortality estimates for the Cape ground squirrel; however, both species of snake share habitat and burrows with Cape ground squirrels and puff adders have been documented consuming both adult and juvenile Cape ground squirrels (J.M. Waterman pers. comm.).

Methods

My field work was conducted at the S.A. Lombard Nature Reserve in the North West Province of South Africa (25°30'E, 27°35'S). The reserve is classified as Kalahari grassland consisting of Cymbopogon–Themeda veldt on a floodplain (Van Zyl 1965). Both species of snake are frequently seen at the field site (Unck et al. 2009) though no population estimates are currently available for the reserve. I live-trapped ten adult squirrels (five males and five females; body mass mean \pm SE = 658.5 \pm 0.78g) from the

study population using Tomahawk live traps (15 x 15 x 50cm; Tomahawk Live Trap Co., Tomahawk, WI, U.S.A.) between June and November 2011. I constrained trapped squirrels in a cloth bag (Koprowski 2002) and collected 1 ml of whole blood via the femoral or caudal arteries using 27-gauge needles and capillary tubes (see Waterman (2002) for more details on trapping and handling methods). The blood was stored in 1.5 ml microcentrifuge vials at 4°C overnight. I discarded the clots before centrifuging the blood sample at 2000 rpm for 30 min at 4°C to separate any remaining erythrocytes. The remaining plasma was stored at -4°C until use (Biardi et al. 2000). Venom from wild-caught puff adders (n=3) and snouted cobras (n=2) was collected (and immediately frozen) in the North West province of South Africa. I mixed the venom into 10mg/ml of 20 mM Tris-HCl+1 mM CaCl₂, pH 8.0 and stored at -20° C at the University of Pretoria until use.

I quantified hydrolysis of gelatin based on methods from Biardi et al. (2000) and Palmer (1993). I made a gel solution of 1.0% agarose and 0.75% gelatin (Type I, bovine skin, Sigma Chemical Co.) in 20 mM Tris-HCl+1 mM CaCl₂ which I poured into 140mm glass petri dishes. I punched equally spaced 3 mm diameter wells into the gel and loaded them with 20 µl of sample. Our three treatments were: (1) 5 µl venom (10 mg/ml) in 15 µl Tris buffer (control); (2) 5 µl venom+5 µl serum+10 µl Tris buffer; and, (3) 5 µl serum+15 µl Tris buffer (second control). I incubated the gel at 37°C for 24 hours and then precipitated the unhydrolyzed gelatin in a saturated ammonium sulfate solution at 70° C for 10 min. I quantified the area of lysis (the breaking down of cells) by averaging two measurements of the diameter across the lysis zone using calipers (Biardi et al. 2000). I replicated each treatment three times per individual for both venoms to increase

precision. I randomized the order of treatments on each plate using a random number generator. I calculated means for the diameters of each treatment and tested their normality using Shapiro-Wilk tests and found all data normally distributed. I compared means using ANOVA, and Tukey-Kramer HSD tests for all treatments (Zar 1999).

Results

When exposed to puff adder venom, the damage in the areas treated with venom and venom-serum were significantly larger than the areas treated with the serum control (Figure 4.1, $F_{2,29} = 4462.21$, $p < 0.001$). I found no significant difference in size of the lysis diameters when I compared areas treated with venom to those treated with venom-serum (Figure 4.1, $F_{1,19} = 0.18$, $p = 0.67$). When I examined the snouted cobra venom hydrolysis of the gelatin, I found the control areas (serum only) to be significantly smaller than both the venom and venom-serum areas of lysis (Figure 4.1, $F_{2,29} = 833.26$, $p < 0.001$). Similar to puff adders, I found no significant difference in diameter when I compared the venom only to the venom-serum treatments (Figure 4.1, $F_{1,19} = 1.20$ $p = 0.28$). Area of lysis diameter was also significantly larger in the puff adder's venom only treatments than the snouted cobra's, illustrating the different proportions of cytotoxic properties in the two species' venoms (Figure 4.1, $F_{1,19} = 1103.00$, $p < 0.001$).

Discussion

The lack of resistance against proteolytic activity in the sera of the Cape ground squirrel against both puff adder and snouted cobra venom differs from the findings of Biardi et al. (2000). They compared a North American sciurid with one sympatric and two allopatric crotalid snakes. Their results indicated that the California ground squirrel's

sera inhibited proteolytic activity of venom from the sympatric species more effectively than two allopatric species. The Cape ground squirrel and the California ground squirrel are similar behaviourally in that both species approach, inspect, and harass venomous snakes that are documented predators of the species (Owings and Coss 1977; Waterman 1997). The risks of harassment are mitigated in California ground squirrels by proteolytic resistance to snake venom, whereas the Cape ground squirrel is risking death from envenomation when approaching within striking distance.

Other mammals that frequently interact with venomous snakes have venom resistance. For instance, several species of opossum (*Didelphis*) that prey on various pit vipers have natural resistances against their venoms (Perez et al. 1979; Oliveira & Santori 1999; Almeida-Santos et al. 2000). Rock squirrels (*Otospermophilus variegatus*), another North American ground dwelling sciurid, also have natural inhibition in their sera against rattlesnake venom's digestive and haemostatic activities (Biardi & Coss 2010). Rock squirrels are similar to California ground squirrels and Cape ground squirrels in their approach and harassment of venomous snakes (Owings et al. 2001). Unlike the California ground squirrel, and similar to the Cape ground squirrel, the rock squirrel is exposed to multiple venomous snakes throughout its range to which it shows multiple resistances (Biardi & Coss 2010). Several mongoose species (Herpestidae) have venom resistances. The Egyptian mongoose (*Herpestes ichneumon*) has strong resistance against venoms from the viperid and elapid families as well as against sarafotoxins, which are unique to the Atractaspididae family (Bdolah et al. 1997). The Indian gray mongoose inhibits haemorrhagic activity against 17 species' venoms (though not puff adders) and has resistance to elapid postsynaptic neurotoxins (Tomihara et al. 1990; Barchan et al. 1992).

Lack of venom resistance could be due to phylogenetic constraints, as the Cape ground squirrel's ecology and behaviour are similar to other resistant sciurids. Two of the three genera in Xerini (*Xerus*, *Atlantoxerus*) are endemic to Africa whereas North American squirrels belong to the tribe Marmotini (Mercer & Roth 2003; Herron et al. 2005; Matthews et al. 2007). Further investigation into venom resistance within the two clades needs to be conducted to understand the extent of proteolytic venom resistance in sciurids. There could be variations in historical snake exposure among *Xerus* populations so further sampling of *Xerus* populations throughout their range is important as well (Coss et al., 1993; Owings and Coss 2008).

In addition, the Cape ground squirrel has a more complex snake predator community than North American ground squirrels. These diffuse evolutionary pressures could hinder specialization in targeting individual venom components from any one venomous species (Thompson 1994). Also, venoms from both snake species tested are complex and usually contain multiple toxin types (Joubert 1976; Broadley 1990; Mallow et al. 2003:). The Cape ground squirrel may have resistance to other types of toxins in the venoms I tested. However, they would still need to minimize the tissue destruction that follows envenomation in order to maintain competitiveness against conspecifics and avoid predation (Biardi et al. 2005). Research on how ground squirrels respond to venom toxins physiologically helps clarify the risks to squirrels in snake-squirrel interactions (Biardi and Coss 2010). The lack of proteolytic resistance in the Cape ground squirrel increases the risk of harassing venomous snakes. Future research could focus on potential reasons for the occurrence of harassment behaviours despite the risk of envenomation.

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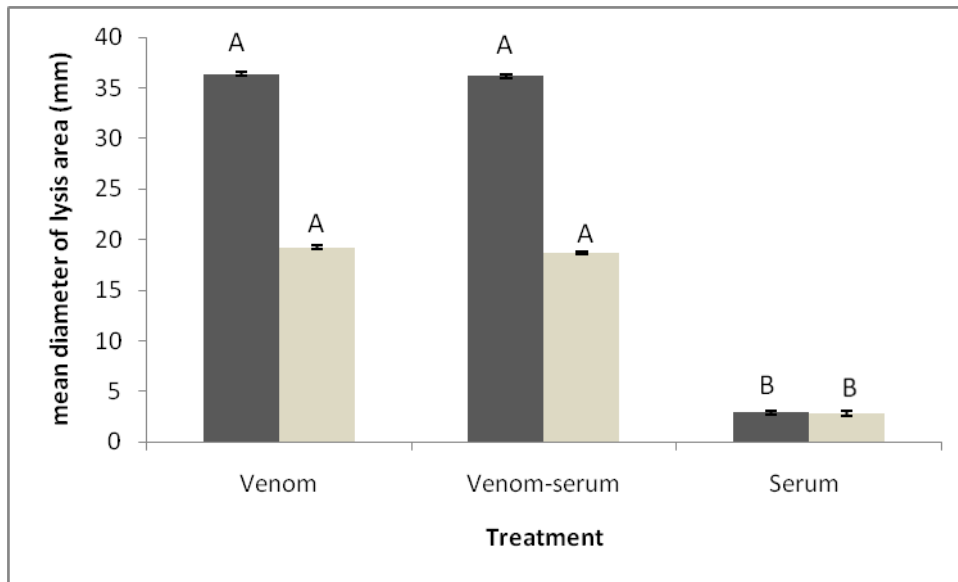
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Figure 4.1: Diameter of area of gelatinase activity caused by pooled venom of puff adders (dark gray) and snouted cobras (light gray). Mean values (\pm SE) are shown for each of the three treatments: venom alone, venom with the sera of Cape ground squirrels and the control. Comparisons for all pairs using Tukey-Kramer HSD shown above each treatment.



Chapter V: Thesis Conclusion

I did not observe equal participation in snake harassment in Cape ground squirrel social groups. When individuals approached a snake during the trial, they either passively inspected the snake or actively harassed it. Inspection is assumed to be less costly than direct harassment, as it probably uses less energy and attracts less attention from the predator, and I found that all sex/age classes in the social group engaged in inspection behaviour. However, harassment behaviour is assumed to be the most costly anti-snake behaviour, because it actively engages the individual with the predator, and females with juvenile offspring present during the trial participated the most in snake harassment.

Both inspection and harassment behaviours increased in total duration with risk; snouted cobras elicited the most individual investment in both activities. Because both behaviours were only elicited by predators or odour cues from those predators, I conclude that both inspection and harassment are predominantly anti-predator behaviours. However, the difference in sex/age class participation between inspection and harassment may occur because the behaviours perform different functions. Close range inspection may give the inspecting individual information about the predator and would therefore be a form of self-defence (Dugatkin & Godin 1992). However, females with juvenile offspring engaged in harassment of snouted cobras the most. Because juvenile Cape ground squirrels are smaller, slower and stay closer to the burrow cluster than adults they are probably more susceptible to predation (Waterman 2002). Active harassment may serve to drive away potential cobra predators from vulnerable offspring and therefore harassment increases the mother's direct fitness by increasing her offspring's survival.

My project also examined the role olfactory cues play in predator discrimination. When presented exclusively with olfactory cues from snakes, Cape ground squirrels reacted as they did to live snakes, harassing only the most dangerous species while increasing inspection and decreasing time spent near snake odours with increased level of threat. The olfactory experiments supported that Cape ground squirrel were capable of discriminating among snake predators by odour cues, differentiating snake odours from neutral odours and between a venomous and non-venomous species.

I found no support for proteolytic venom resistance in the Cape ground squirrel. I conclude that the costs of approaching and harassing the two venomous snake species used in this study (snouted cobra and puff adder), as well as any species with cytotoxic venom components, include a significant mortality risk along with the energetic and opportunity costs to the individual (Dugatkin & Godin 1992).

Previous research conducted on another population of Cape ground squirrels using tethered puff adders (see Owings et al. 2001 for details on this method; J.M. unpublished data) in Namibia (10 trials in 1996 and 3 trials conducted in 2005) found a different pattern in sex/age class harassment participation; adult females and dispersed males harassed in similar proportion. There are multiple differences between the Namibian and South African populations including variation in the social and mating systems, and population dynamics in the squirrels, as well as environmental differences like vegetation and rainfall between these two populations (Waterman 1996; Pettitt et al. 2008; Scantlebury et al. 2008; Manjerovic 2010). Further research could be conducted examining how resources and social structure influence predator harassment by conducting a comprehensive comparison among Cape ground squirrel populations. Also,

by quantifying the energetic and opportunity costs of mobbing snakes we could better understand the total costs of snake predator harassment in Cape ground squirrels.

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