Both familiarity and kinship influence odour discrimination by females in a highly social African ground squirrel

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Highlights

• We tested female kin discrimination abilities in a highly social ground squirrel.

• On a coarse scale, female investigations of kin and nonkin odours did not differ.

• Degree of relatedness influenced investigation duration only with strangers' odours.

• Both familiarity and degree of relatedness affected kin discrimination.

Abstract

Kin recognition can be important in species where inbreeding avoidance or nepotism (favouritism towards kin) rely on identifying kin, particularly in species with alloparental care. The mechanisms that facilitate kin discrimination, where recognition is determined through cues that correlate with relatedness, usually include either prior association (familiarity) or phenotype matching or both. Odour is an important cue used in a number of mammalian species to discern kin, particularly the ground-dwelling squirrels. Cape ground squirrels, *Xerus inauris*, are a cooperative-breeding species, living in tight-knit family groups. However, group fission, promiscuity and the large home ranges of breeding males result in a

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high variance in relatedness both within and among social groups, making them a good species to investigate kin discrimination. We examined whether females are capable of discriminating between the odours of familiar versus unfamiliar females that varied in their relatedness to the focal female using odour experiments. Overall, the average duration of sniffing of the odours of familiar and unfamiliar females did not differ. Similarly, females did not adjust their sniff duration relative to the degree of relatedness of familiar females. However, females appeared to discriminate by the degree of relatedness of unfamiliar (stranger) females, spending longer sniffing odours from females that were not related to them. Thus, females were capable of discriminating the degree of relatedness from odour but they did not do so within their family group. We conclude that Cape ground squirrels are able to discriminate kin. However, whether females in this facultative cooperative breeder use the degree of relatedness in direct social interactions and nepotistic behaviours remains to be investigated.

Kin recognition occurs when an individual recognizes another individual as genetically related kin, allowing for discrimination at a genetic level (Beecher 1982; Stuart 1991). The ability to distinguish kin from non-kin individuals is advantageous for a number of reasons; discrimination of strangers from kin may help avoid inbreeding (Harvey and Ralls 1986; Leclaire et al. 2013; Gilad et al. 2016) and protect territory as well as pups from agonistic strangers (the 'dear enemy phenomenon' and 'threat level' hypotheses) (Temeles, 1994; Cross et al, 2013). Kin recognition may also aid in kin selection and the promotion of nepotism (Mateo 2002).

Kin selection is the most common explanation of the evolution of cooperative breeding (Griffin & West 2003). Recent studies on the evolution of cooperative breeding have suggested monogamy is critical to the evolution and maintenance of cooperative breeding, as this mating system ensures relatedness between helpers and breeders stays high, a critical requirement for helpers to benefit via kin selection (Lukas & Clutton-Brock 2012a,b). However, even in promiscuous breeding systems, helpers could gain inclusive fitness benefits by using kin discrimination to bias their care to close kin (Cornwallis et al. 2009; Kramer & Russell 2014). This latter hypothesis assumes that individuals can discriminate kin from non-kin, and predicts that kin discrimination is critical in species where relatedness varies, such as in cooperative breeding species with promiscuity (Cornwallis et al. 2009; Kramer & Russell 2014).

Kin discrimination has been widely documented in mammals, usually through the mechanisms of familiarity and phenotype matching (Mateo 2002, 2003; Breed 2014; Smith 2014). Familiarity or shared association allows kin discrimination when interactions amongst kin are predictable, such as sharing nests or home ranges (Mateo

2003). Through such exposure, particularly during critical times of development, individuals will become familiar with their kin's individual cues and recognize them in different locations or across time (Mateo 2002; Breed 2014; Smith 2014; Nichols 2017). Individuals may also identify kin through the shared association of another individual with a close relative (e.g., mother and non-littermate siblings; Smith 2014). Familiarity or shared association as means of kin recognition is important when individuals are philopatric and remain near kin (Mateo 2003). However, in some situations, kin may interact without any prior association, using other means to identify each other, such as self-referent phenotype matching (Holmes & Sherman, 1982). In phenotype matching, individuals discriminate kin they have never met by comparing recognition cues to their own learned recognition template (Holmes & Sherman 1982; Holmes 1986; Mateo 2003). This type of recognition allows the individual to determine not only who is kin or not, but also more refined assessment of their degree of relatedness (Mateo 2002). Phenotype matching is expected to be an important mechanism in situations where unfamiliar kin are likely to be encountered, such as in species where males move or disperse over large areas, mating with multiple females (permitting the recognition of paternal as well as maternal relatives; Mateo 2003; Widdig 2007), or where kin of different ages may meet without association cues (Holmes & Sherman 1982; Mateo 2003). Promiscuity, in particularly, may drive kin recognition via self-referent phenotype matching (Hain & Neff 2006 but see Hain et al. 2016).

Nepotistic behaviours are important components of sociality in the ground-dwelling sciurids, including the sharing of burrows, alarm calling and patterns of tolerance (Mateo 2003; Holmes & Sherman 1982; Hare 2004). High interspecific

variance in the structure of sociality, degree of philopatry and mating systems in the sciurids have been the focus of a number of studies on sociality and nepotism (Armitage 1981; Mateo 2003; Blumstein & Armitage 1999). Not surprisingly, research on kin recognition has also been extensive, with both discrimination via prior association or phenotype matching (or both) documented in social and asocial species (Mateo 2002; 2003; Hare 2004).

Both mechanisms of kin discrimination help an individual recognize if another individual is kin before responding appropriately (Breed 2014; Mateo 2017; Nichols 2017). The actual cues used to identify kin vary, depending on species, but can include visual, auditory, and odour cues (Beecher 1982). Communication by scent and scent marking is a major means of kin discrimination in mammals and particularly in ground squirrels (Mateo 2003, Shave & Waterman 2017). Belding's ground squirrels (*Urocitellus beldingi*) use odours from the oral, dorsal, pedal, and anal glands for kin recognition (Mateo 2006). Mateo (2002, 2006) found that Belding's ground squirrels spent longer investigating cubes with odours of non-kin, and were able to discriminate the scent of unfamiliar distant kin ($r < 0.125$). This species appears to use both prior association and phenotype matching, while a number of other ground squirrels appear to use only prior association (Hare & Murie 1996; Hoogland 1995; Mateo 2003). Columbian ground squirrels (*Urocitellus columbianus*) have been found to use scent to discriminate between familiar and unfamiliar conspecifics (Harris & Murie 1982), but do not appear to use phenotype matching (Hare & Murie 1996). Similarly, prior association was the main mechanism of discrimination in yellow-bellied marmots, black-tailed prairie dogs, and thirteen-lined ground squirrels (Armitage 1989; Holmes 1984;

Hoogland 1995). Mateo (2003) points out that these latter studies used analyses of social interactions and she suggests tests using odour perception may be a better means of determining the mechanisms of kin discrimination.

Kin recognition may be important in the highly social Cape ground squirrel (*Xerus inauris*). Unlike the majority of North American ground squirrels that do not live in cohesive groups with other adults (Michener 1983), Cape ground squirrel females live in matrilineal kin groups that share a burrow system, where they sleep, forage, and reproduce communally (Waterman 1995). They are cooperative breeders with alloparental care and a promiscuous breeding system (Waterman 1998; Pettitt & Waterman 2011, Manjerovic & Waterman 2015), a rare social system in ground squirrels (Blumstein & Armitage 1999) and in mammals overall (Russell 2004). While males disperse from their natal groups, females are philopatric, staying in their family groups for their lifetimes or budding off with a family member to form a new group adjacent to their old one (Waterman 1995, 2002). Because of group fission, maternally related individuals may be located in adjacent social groups (Waterman 2002). Males roam widely in all-male bands and seek out females in oestrus, who mate with multiple males in a single oestrous period (Waterman 1998). Male reproductive success is also strongly skewed, with only 28% of males siring offspring (Manjerovic & Waterman 2015). As a result of this reproductive skew and promiscuity, individuals within and among different family groups are more likely to be related paternally, adding to the variance in relatedness across the landscape.

Odour appears to be an important means of communication in female Cape ground squirrels. They scent mark in a variety of ways, including by rubbing their snout

or anal region on objects and other squirrels, and will investigate the scent marks of other squirrels (Straschil 1975). Adult females spend more time investigating the odour of unrelated (dispersed) males compared to related (family group) males but do not appear to discriminate by finer degrees of relatedness (Shave & Waterman 2017). However, we know little about the use of odour in the recognition of female kin and non-kin conspecifics in this facultative cooperative breeder.

The high intra- and intergroup variance in Cape ground squirrels, along with their cooperative breeding, make them an excellent species in which to examine kin discrimination. In this study, we examined whether Cape ground squirrels discriminate between related and unrelated individuals by odour. We tested the hypothesis that female Cape ground squirrels could discriminate between familiar female groupmates and unfamiliar female strangers by odour alone, predicting that if discrimination by scent did occur, then the odours of unfamiliar females would elicit longer durations of investigation than familiar animals. We also tested the hypothesis that discrimination is influenced by more than just familiarity, but also by a finer scale of relatedness using phenotype matching, predicting that females would alter their duration of investigation based on varying degrees of relatedness within family groups and with unknown females.

Methods

Biology of the Study Animal

Female Cape ground squirrel groups consist of 1-5 adults along with 2-3 sub-adults of either sex (Waterman 1995; Hillegass et al. 2008). Female groups live in burrow clusters (aggregations of burrows) clearly separated by adjacent burrow clusters and only one

family group lives in a burrow cluster (Waterman 1995). Females within a social group have no obvious dominance hierarchy, show no territoriality and are highly amicable compared to other species of ground squirrels (Waterman 1995; Pettitt & Waterman 2011). Amongst members of their own social groups, female squirrels frequently interact with familiar groupmates and display cooperative behaviours including allogrooming and alloparental care (Waterman 1995; Pettitt & Waterman 2011). However, there is evidence of competition within groups, as groups that get too large will eventually split, with some females moving into adjacent areas to their natal group (Waterman 2002). Interactions among females of adjacent social groups are rare, and any interactions are usually agonistic (Waterman 1995). Some mature males delay dispersal from their family groups until they are two to three years old, but eventually all males disperse and join all-male bands composed of dispersed males (Waterman 1995; Manjerovic & Waterman 2015). The home range of dispersed males is much larger than females (7 times the size in a population in Namibia; Waterman 1995), and they overlap with numerous family groups (Waterman 1995). Mating occurs year-round, is asynchronous among females within and between groups (Waterman 1996). Breeding is highly promiscuous, with an average operational sex ratio of 11 males to a single female, and females mate with an average of four males (Waterman 1998; Manjerovic & Waterman 2015). Although litter size is small (1 to 2 offspring), 90% of litters of two are multiply sired (Manjerovic & Waterman 2015). Adult females isolate from their social group during lactation, returning to their family group with their offspring within a day or two after emergence of their offspring (Waterman 1996).

Study area, trapping and handling

The study was conducted between July 7- July 21, 2013, and June 11- July 30, 2014, at S.A. Lombard Nature Reserve in the North West Province of South Africa (25°30ʹE, 27°35ʹS). We have studied Cape ground squirrels at this site since 2002, allowing us to track and identify existing family groups before this study occurred (Hillegass et al. 2008; Pettitt & Waterman 2011).

We trapped 29 adult non-pregnant females using Tomahawk live traps (15 x 15 x 50 cm; Tomahawk Live Trap Co., Tomahawk, WI, U.S.A.) baited with a mixture of birdseed and peanut butter. We collected data on the mass, sex and reproductive condition of individuals while they were in cloth handling bags, which minimize contact and decrease stress (Koprowski 2002; Unck et al. 2009). We used six different handling bags during the experiment but it was not feasible to have a unique handling bag for each squirrel. Thus, residual chemical cues may have been transferred between trapping locations (Hare 1994). We assessed the reproductive condition of each female by noting vulva swelling to detect oestrus. We assessed nipple status (nipples swell permanently when females start breeding; Pettitt & Waterman 2011) and palpated the abdomen to detect the presence of foetuses to determine pregnancy (Shave & Waterman 2017). For permanent identification, we implanted each individual with a passive integrated transponder (PIT tag; AVID Inc., Norco, CA, USA) and all individuals were marked with a unique dye mark (Rodol D; Lowenstein and Sons, New York, NY, U.S.A.) so they could be identified from a distance. We also collected a small amount of skin from the tip of the tail (< 3 mm) for genetic analysis (see below). We released females at their site of capture.

Odour trials

We used twenty reproductively mature (adult) non-pregnant female squirrels as sources of odours in our trials. Three females were used twice as unfamiliar odours but new scents were collected before each trial. We collected odours from the circum-oral area. Scent from the oral region (which contain large apocrine glands), is involved in direct social interactions in many ground squirrels, particularly behaviours involved in greeting (Kivett et al. 1976; Mateo 2003). We chose the oral gland area because this gland is used in scent marking in Cape ground squirrels (Straschil 1975). In addition, greeting behaviours (naso-oral contact) and approaches (one squirrel walking to within 10 cm of another individual, face to face) are common behaviours within and between family groups in this species (Waterman 1995). To collect samples, we constrained a squirrel in a cloth bag. We gently rubbed each facet of a polyethylene cube (¾ x ¾ x ¾ cm, Acryl Design Ltd. Winnipeg, MB, Canada) three times on the oral region of the squirrel's face (Mateo 2006). We used new latex gloves when handling each odour cube to avoid contamination. We then placed the odour cubes in individual plastic bags in a cooler with ice packs and used the cube in a trial immediately or we stored the cube at -20°C and used it within 24 hours of collection (Raynaud & Dobson, 2011; Shave & Waterman 2017). For each odour trial, we used an unscented cube as a control. After use, the cubes (and tarps, see below) were soaked for five minutes in a 50% vinegar solution, rinsed with water, and dried by sunlight before being used again (Phillips & Waterman 2013; Shave & Waterman 2017).

We presented odours to 12 focal adult non-pregnant females from 12 different social groups. Target females were presented with three odour cubes simultaneously: one with odour from a familiar female (collected from an adult female squirrel in the same

social group as the focal individual), one from an unfamiliar female (collected from a distant and different social group), and an unscented control cube. We feel confident that the focal females and unfamiliar females had never met prior to conducting our experiment. The average home range of a female Cape ground squirrel at our South Africa site is 1.1 ha (Lem, unpubl. data) and the distance between adjacent social groups is 69.9 ± 8.4 m (mean ± SE; Scantlebury et al. 2008). Thus the distance between the location of focal females and the unfamiliar female locations (mean ± SE = 1549.13 ± 178.7 m; range = 623.7-2749.9 m, N = 12) was far greater than the normal movements of females.

We conducted odour experiments as soon as we collected both odours. To initiate a trial, and ensure we had the correct focal animal, we walked towards a focal female until she immerged into a burrow. We then placed a clean 15 x 50 cm polypropylene tarp 1.0 m away from the burrow entrance where the focal female had immerged. The odour cubes were placed 15 cm apart from each other on the tarp such that each cube was an equal distance from the burrow entrance (Shave & Waterman 2017). We also placed 2 ml of peanut butter 10 cm in front of each cube to encourage the focal squirrel to approach the tarp after emerging from the burrow (Shave & Waterman 2017). We randomized the order of cubes on the tarp for each trial by drawing cube locations out of a hat, but the observer was not blind to this order.

We performed odour trials opportunistically throughout the day (between 0800 h and 1700 h local time). We observed the females from hides on top of vehicles or from towers or similar structures (at least 50 m away from the cubes), using 10 x 50 binoculars and spotting scopes (20-45 x 60) (Bushnell Co., KS, USA). We used the Voice Memo

app in an iPod Touch (IOS v. 6.1.6, 16-GB 4th Generation, Apple Inc., California, USA) to record of the observer's observations of the squirrel's behaviour (Phillips & Waterman 2013; Shave & Waterman 2017). Examination of odours by focal individuals was measured as the duration of time (s) the squirrel spent sniffing each cube (the female approached within 5cm of the cube and visibly twitched the nose and whiskers; Cross et al. 2013; Phillips & Waterman 2013; Shave & Waterman 2017) by recording the elapsed time on the Voice Memo app time stamp during playback. We observed for 30 minutes or until the focal squirrel left the cubes for longer than 5 minutes. We also recorded wind and weather conditions to ensure that wind did not affect squirrel behaviour or odour dispersal; trials were not run if wind speeds were higher than 18.0 km/h (Phillips & Waterman 2013; Shave & Waterman 2017).

Microsatellite DNA

We used tissue samples (skin) collected from tail tips to determine the coefficient of relatedness (r) between the focal female and the familiar and unfamiliar odour donors using molecular markers. We extracted DNA using a E.Z.N.A ® Tissue DNA Kit (Omega Bio-tek, Inc., Norcross, GA, USA) and all individuals were genotyped using 20 species-specific microsatellite loci developed for this species (Abercrombie et al. 2009; Manjerovic & Waterman 2015; Shave & Waterman 2017). Polymerase chain reaction (PCR) and cycling parameters are described in Abercrombie et al. (2009). All amplified DNA was sequenced at the Centre for Applied Genomics (Hospital for Sick Children, Toronto, Canada), and we scored each allele using Genemarker (v.2.6.0). Of the 29 individuals used in trials (focal females or odour donors), 27 were typed at $\geq$ 15 loci and two were genotyped at 11 and 14 respectively. Previous studies have found eight loci

have adequate polymorphic information content to determine relationships in this species (Manjerovic & Waterman 2015). We used the triadic likelihood estimator in COANCESTRY (v1.0.1.8; Wang 2011) to estimate pairwise relatedness using permutations of 10,000 bootstraps (Wang 2007, 2011) as this estimator has the highest correlation with our data (Shave & Waterman 2017). The coefficient of relatedness (r) values generated from COANCESTRY range from zero to one, where zero represents dyads that do not share alleles by common descent and a value of one means the dyads shared all alleles by common descent. We did not know the degree of relatedness among individuals prior to or during the odour trials.

Data analysis

As we could not normalize the sniff duration data, we used a non-parametric two-way (Friedman's) ANOVA, blocking on focal female ID, to test for differences between the total time spent sniffing for familiar, unfamiliar and control cubes and then used posthoc comparisons tests (Wilcoxon method) for pairwise analyses. We used Cohen's d-scores as a measure of the effect size of the latter comparison. We used Spearman's correlations to examine sniff duration and coefficients of relatedness. As coefficients of relatedness values were normal, we used a Welch's t-test for unequal variance to compare the relatedness of the focal female to groupmate versus unfamiliar females. We used JMP V.12 (SAS Institute Inc., Cary, NC, USA) for all statistical analyses. A 0.05 probability of a Type I error was considered significant. Results are expressed as mean ± standard error (SE).
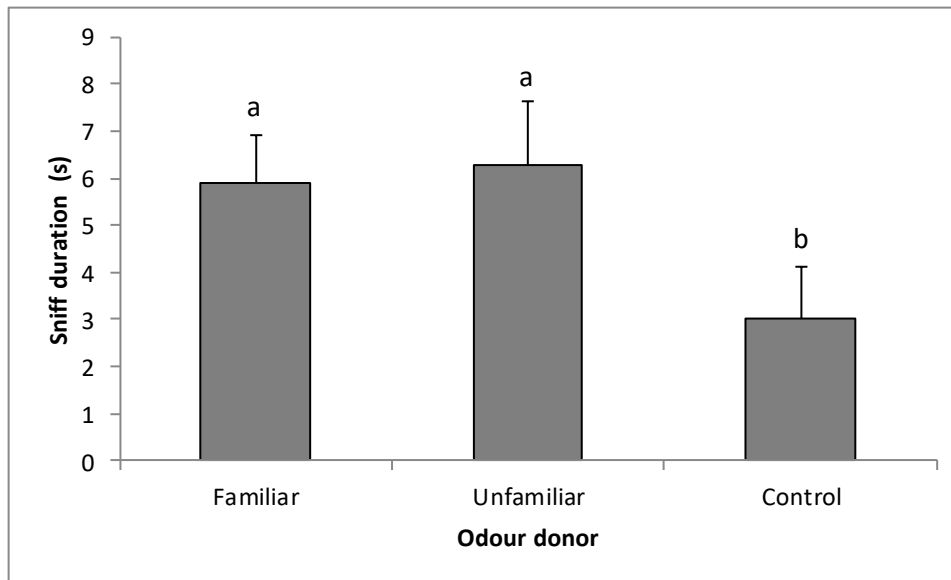
Figure 1. Differences in duration (seconds) spent sniffing by female Cape ground squirrels in response to different odours (familiar and unfamiliar female odour and an unscented control; N = 12 for each treatment). Mean values (±SE) are shown for each of the three treatments. Treatments not connected by the same letter are significantly different (P < 0.05).

Results

During the trials, all 12 females approached within 10 cm of all three cubes, but there was no difference in which cube was approached first (control, 50% (6); familiar, 25% (3); unfamiliar, 25% (3); Chi-square test, $X^2_{(2)} = 1.50$, $P = 0.47$). There was also no difference in time spent within 10 cm of each cube (mean duration familiar cube 29.8 ± 5.69 s; unfamiliar cube 29.25 ± 9.37 s; control cube 27.5 ± 10.08 s; Friedman's test, $X^2_{(2)} = 2.40$, $P = 0.30$). The duration that females spent sniffing the odour cubes differed (Friedman's test, $X^2_{(2)} = 6.52$, $P = 0.03$; Figure 1), with females spending less time sniffing the control cube compared to the odour of familiar (Wilcoxon multiple comparisons test, $Z = -2.11$, $P = 0.035$) and unfamiliar females ($Z = -2.23$, $P = 0.026$). The duration of sniffing between familiar and unfamiliar female odour cubes did not differ ($Z = 0.029$, $P = 0.98$). The statistical power of both the comparisons of familiar with control and unfamiliar with control were high (0.88 and 0.87 respectively). The comparison of sniff duration between the familiar and unfamiliar had lower power (0.07) but we would need a sample size of over 1050 to achieve statistical power > 0.80.

Focal individuals were more closely related to familiar (donor groupmate) animals (0.28 ± 0.06, range 0 to 0.62) than unfamiliar donor animals (0.04 ± 0.01, range 0 to 0.12; paired t-test, $t_{(11.9)} = -3.73$, $P = 0.003$). The duration of time a female spent sniffing a cube was not correlated to the coefficient of relatedness (r) in the familiar (groupmates) treatment (Spearman's rank correlation: $r_s = -0.021$, $N = 12$, $P = 0.95$) but was negatively related to kinship when females were exposed to unfamiliar individuals ($r_s = -0.74$, $N = 12$, $P = 0.0058$; Figure 2). We also examined if the difference between the relatedness of the two cubes influenced female behaviour. We did not find a relationship between sniff duration and such a difference in the coefficients of relatedness
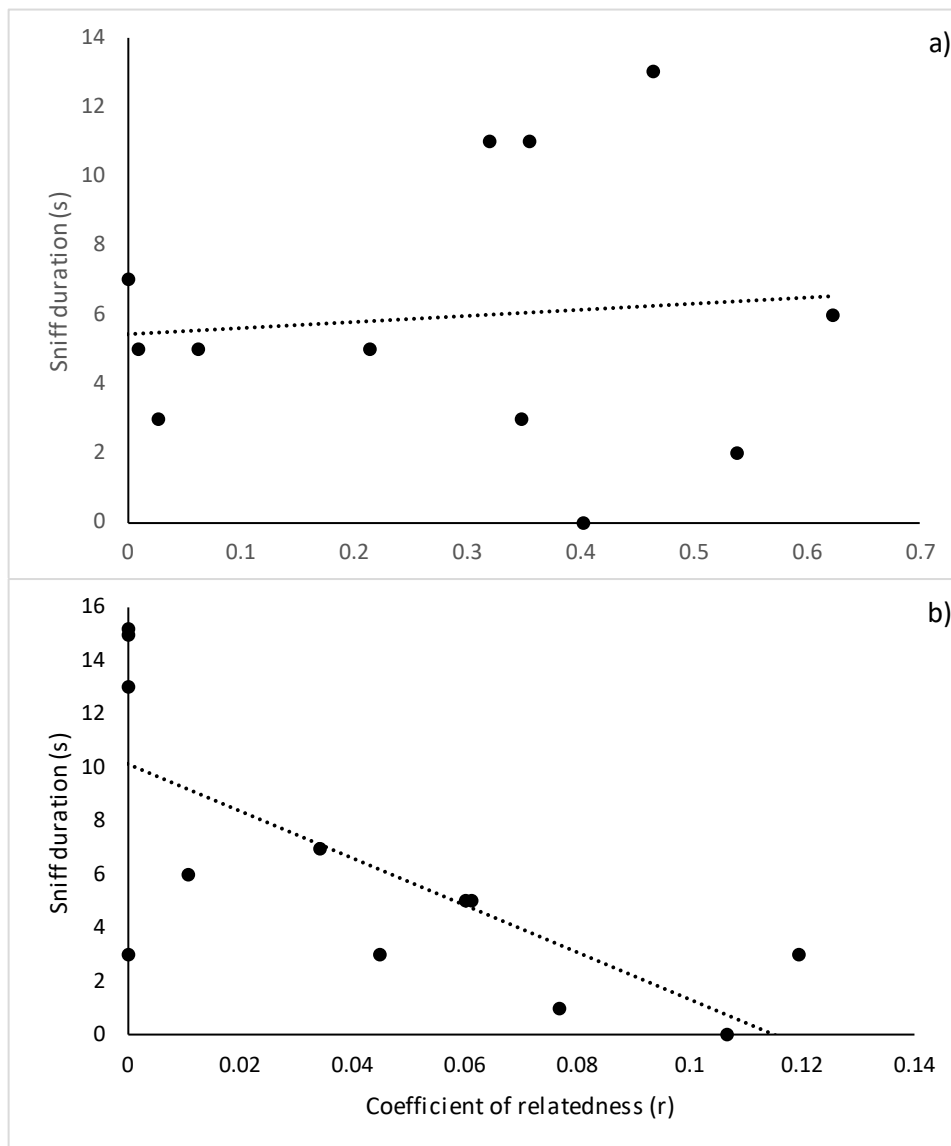
Figure 2. Duration of time spent sniffing cubes by female Cape ground squirrels (N = 12) in relation to the genetic relatedness (r) of odours from familiar (a) or unfamiliar (b) adult females.

(familiar sniff duration vs difference in coefficient, $r_s = -0.102$, $N = 12$, $P = 0.75$; unfamiliar sniff duration vs difference in coefficient of relatedness, $r_s = 0.32$, $N = 12$, $P = 0.31$).

Discussion

We found that female Cape ground squirrels appeared to detect the oral gland odour of conspecifics, as they approached all cubes (within 10 cm) but still spent less time sniffing the unscented controls, suggesting a response to the scents of conspecifics and not just the presentation of a novel stimulus. These squirrels are known to investigate the scent marks of conspecifics (Straschil 1975) and they respond differently to male odour based on familiarity (Shave & Waterman 2017). However, they showed no overt difference between the odours of familiar versus unfamiliar females. We also did not find a relationship between duration of sniffing the scent of family members and degree of relatedness, but females did appear to discriminate on the degree of relatedness of females they had never met, spending more time sniffing the odours of females that were unrelated to them. Thus although we did not detect differential investigation overall, the squirrels did investigate the cubes differently, adjusting their time spent sniffing in relationship to kinship only with unfamiliar odours.

Our results were surprising for two reasons. First, family groups of Cape ground squirrels vary in relatedness because of the high degree of promiscuity, and the relatedness of familiar scents to the responders ranged from 0 to 0.62. Thus, we expected that females would differentially investigate odours from within their own family group based on degree of relatedness, similar to findings by Hain & Neff (2006) in bluegill

sunfish (*Lepomis macrochirus*). Second, when females are exposed to the anal scent of

groupmate (non-dispersed) and unfamiliar males, they clearly sniffed the unfamiliar

males longer than that of familiar males, but they did not discriminate by degree of

relatedness for either group of males (Shave & Waterman 2017). The difference between

the results from the latter study and the current could be because different information is

encoded in the different scent glands (Mateo 2006; Cross et al. 2013; Reynaud & Dobson

2011) or because it may be more important for females to discriminate the scent of males

on a more general basis (based on shared association) to avoid inbreeding with close

relatives.

Odour discrimination by mammals can be explained by the "dear enemy

phenomenon", the "threat hypothesis" or for nepotistic reason (kin selection) (Coss et al

2013; Raynaud & Dobson 2011). Both the "dear enemy phenomenon" and the threat

level hypotheses predict discrimination between familiar neighbour and stranger

conspecifics in territorial species that adjust their aggression depending on the probability

of losing their territory (Temeles 1994; Raynaud & Dobson 2011). Neither of these

hypotheses are applicable to Cape ground squirrels, as males or females are not territorial

and agonistic interactions are extremely rare (Waterman 1995; Pettitt & Waterman 2011).

The discrimination of kin versus non-kin has been supported in vertebrates and

invertebrates and has been the focus of a number of reviews (see Tang-Martinez 2001;

Mateo 2003; Breed 2014; Smith 2014). Many studies examining kin recognition by scent

in mammals have found a difference in the behaviour of focal individuals to familiar and

unfamiliar scents, including marsupials (Blumstein et al. 2002, Charlton 2015),

carnivores (Leclaire et al. 2013, Gilad et al. 2016, Mitchell et al 2018) and numerous

ground squirrel species (Raynaud & Dobson 2011; Mateo 2002, 2003; Harris & Murie 1982). The majority of these studies have supported a general kin discrimination based on prior association (see Breed 2014 and Smith 2014, as well as Mateo 2003). Despite the potential importance to kin selection of kin discrimination, we found no overt discrimination between the duration of sniffing of familiar versus unfamiliar odours in Cape ground squirrels, and similar lack of general discrimination between kin and non-kin has been found in female yellow-bellied marmots (*Marmota flaviventris*), another species with matrilineal kin structure (Cross et al. 2013).

Some studies have found their species used both prior association and phenotype matching (Vallavicencio et al. 2009, reviewed in Mateo 2002, 2003). However, the ability to discriminate amongst kin according to relatedness does not mean that an individual will actually discriminate in their social interactions (Mateo 2003; Villavicencio et al. 2009; Mitchell et al. 2018). Belding's ground squirrels are capable of discriminating amongst kin in experiments that included response to scent, but such recognition was not reflected in their treatment of kin (Mateo 2003). Degus (*Octodon degus*), although they appeared to be able to discriminate kin by odour, they do not appear to use kin discrimination when interacting with familiar animals (Villavicencio et al. 2009). The lack of general response of female Cape ground squirrels to the scent of female conspecifics provides evidence that this non-aggressive, highly social species interacts amicably with conspecifics, regardless of kin relationship. Yet the ability of the Cape ground squirrels to detect fine scale differences in kinship suggests they are capable of discriminating among kin through phenotype matching. Similar abilities have been seen in suricates (*Suricata suricatta*) in determining mate selection, and house mice (*Mus*

musculus) when choosing nest partners (Leclaire et al. 2013; Green et al. 2015). Likewise, banded mongoose discrimination varies with degree of relatedness, but contrary to our results, their discrimination is only evident with familiar, within group, individuals (Mitchell et al. 2018). Our results suggest that female Cape ground squirrels are able to discriminate between kin and non-kin without previous exposure to the individual or their scent. They may use phenotype matching to discriminate amongst strangers but do not appear to do so with their family. Instead, they may just use a general rule of thumb, similar to other cooperative breeders, that all the familiar animals in their social group are kin (Nichols 2017).

Mateo (2003) points out it is important to use a number of different experiments to exam kin discrimination, including odour trials, cross-fostering experiments, and social interactions. Unfortunately, the asynchronous breeding of Cape ground squirrels within and between social groups (Waterman 1998), does not lend itself to cross-fostering experiments. The next step in our study is to examine, in more depth, social interactions between kin and non-kin within and between social groups to determine if nepotism occurs, particularly if alloparents bias their care towards close kin. Future studies examining differences in scent gland information are also warranted (Mateo 2006).

In conclusion, female Cape ground squirrels did not appear to discriminate overtly between the odours of familiar and unfamiliar female conspecifics, but they did respond differently to family group females and unfamiliar females in terms of degree of relatedness. While they did not appear to use degree of relatedness in investigating the odours of their family group but they did appear to use degree of relatedness to discriminate amongst the odours of unfamiliar, unrelated, individuals. This latter result

suggests that they are able to discriminate kinship based on scent alone (via phenotype matching) but may not use such discrimination with members of their own social group.

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