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ORIGINAL ARTICLE

The effects of familiarity and reproductive status on olfactory discrimination by female Cape ground squirrels (*Xerus inauris*)

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Abstract

The ability to differentiate among conspecifics on the basis of genetic relatedness can be advantageous in kin and mate selection. Selection of mates that are of an intermediate degree of relatedness maximizes the propagation of shared genes to offspring, while minimizing the accumulation of deleterious recessive alleles that can result from close inbreeding. Odors produced from various glandular surfaces of the body have been shown to influence discrimination of conspecifics in numerous taxa, including the order Rodentia. We investigated whether female Cape ground squirrels (*Xerus inauris*) discriminate between the odors of familiar and foreign males by conducting scent experiments. We simultaneously presented odors that had been collected on acrylic cubes from the anal gland of a familiar (non-dispersed) and a foreign male as well as an unscented control, and recorded the response of focal pregnant and non-pregnant females ($n = 19$). Non-pregnant females responded differently to the odors of the scent cubes, sniffing the scent collected from the foreign male significantly longer than either the familiar male or control odor, whereas there was no difference in the response of pregnant females. We conclude that male familiarity may influence female mate choice, as familiarity is reliably correlated with relatedness in this population. Based on prior knowledge about the social behavior of Cape ground squirrels, the ability to discriminate between familiar and foreign males may be used by females as an inbreeding avoidance mechanism, as close inbreeding can be avoided by mating with foreign males.

Significance statement

Olfactory cues are one method of social communication among conspecifics, and can play an important role in nepotism, territory defense, and mate choice. We studied olfactory discrimination in the Cape ground squirrel, a highly social, semi-fossorial mammal that occurs in South Africa. We assessed the response of pregnant and non-pregnant females to the odors of familiar and unfamiliar males. We found that non-pregnant females displayed a preference for unfamiliar males, whereas pregnant females displayed no preference. Cape ground squirrels are non-territorial and exhibit low levels of intraspecific aggression; therefore, our results indicate that odor may be involved in mate selection by females, rather than territoriality, as relatively low levels of inbreeding are maintained in this population.

Keywords Olfactory discrimination · Cape ground squirrels · Familiarity · Relatedness · Mate choice

Introduction

Social recognition, which is generally defined as the ability to distinguish one conspecific from another, has evolved in a variety of taxa as a means to facilitate communication between conspecifics (Gheusi et al. 1994; Brower and Nye 1996; Sherman et al. 1997). The level at which social recognition functions varies within and between species, from discrimination on the basis of familiarity to sex-specific discrimination and the use of genetically mediated cues to differentiate conspecifics (Clarke and Faulkes 1999; Milinski 2006). As well,

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social recognition is most likely context dependent, meaning that the underlying ecological situation influences the level of recognition used by the individual (Zheng et al. 2013). For example, in some territorial species such as the Eurasian beaver (*Castor fiber*), familiarity-based social cues are used to avoid territorial conflict with familiar rivals (Fisher 1954; Rosell and Bjørkøyli 2002).

One level of social recognition that has been observed in numerous taxa is the differentiation between kin and non-kin. Kin recognition allows discrimination among conspecifics on the basis of genetic relatedness, which has direct impacts on kin and mate selection (Smith 1979; Beecher 1982; Harvey and Ralls 1986). Kin selection occurs when there is selection for behaviors or traits that enhance the fitness of related individuals, and is one of the primary mechanisms used to explain apparent altruism and cooperative behavior (Hamilton 1964; Tang-Martinez 2001; Clutton-Brock 2002; Griffin and West 2003). In addition to kin selection, kin recognition can be used to reduce the frequency of close inbreeding when choosing mates (Harvey and Ralls 1986; Leclaire et al. 2013). Inbreeding depression, which results from the mating of two closely related individuals, can lead to the accumulation of deleterious recessive alleles (Harvey and Ralls 1986). These recessive alleles can cause a reduction in the pre- and postpartum viability of inbred offspring and the reproductive success of inbred adults, ultimately reducing the fitness of these individuals (Margulis 1998). The ability to differentiate between kin and non-kin is often seen as an evolutionary advantage (Harvey and Ralls 1986). However, relying too extensively on outbreeding may reduce fitness as well, as a result of losing shared genes between mating individuals that increase inclusive fitness (Barnard and Fitzsimons 1989). The optimal strategy appears to be to select mates of an intermediate relatedness in order to maintain heterozygosity, while maximizing the proportion of shared genes propagated to future offspring (Peacock and Smith 1997; Kokko and Ots 2006).

There are several mechanisms promoting social recognition of conspecifics, including spatial cues, familiarity, phenotype matching, and recognition alleles (Holmes and Sherman 1983; Mateo 2003). Social recognition, particularly familiarity-based mechanisms, can be subject to errors of false inclusion (considering familiar non-kin as relatives) and false omission (excluding kin when they are unfamiliar), and therefore kin need to be reliably associated in both space and time for this mechanism to work (Holmes and Sherman 1983; Wikberg et al. 2014). To more reliably identify kin versus non-kin, recognition alleles can be used to discriminate among conspecifics (Green et al. 2015). Specifically, alleles of the major histocompatibility complex (MHC) are not only involved in immune recognition, but also influence mate choice in house mice (*Mus musculus*) (Yamazaki et al. 1980; Potts et al. 1991; Potts and Wakeland 1993). The polymorphic loci of the MHC strongly influence individual odor production, which allows individuals to differentiate the relatedness of conspecifics through scent (Milinski 2006).

Discrimination of conspecifics using olfactory cues has been widely observed in a variety of taxa (insects: Jaisson 1991; amphibians: Waldman 1991; mammals: Halpin 1986; Johnson 1973; fish: Olsen et al. 1998; birds: Bonadonna and Sanz-Aguilar 2012) and specifically ground-dwelling squirrels (Halpin 1984). Mateo (2006) presented odors from the oral, dorsal, pedal, and anal glands as well as from the ears and urine that had been collected on acrylic cubes from conspecifics to Belding's ground squirrels (*Urocitellus beldingi*) and found all but the urinary odors were used for kin recognition. Olfactory discrimination of conspecifics can function in protection from infanticide, territory defense, and even mate selection (Harris and Murie 1982, 1984; Raynaud and Dobson 2011). Furthermore, the discrimination of conspecifics can be influenced by reproductive state, particularly the discrimination of mates by females (Mossman and Drickamer 1996; Keller et al. 2009; Frynta et al. 2010). In house mice, estrous females showed strong preferences for the odors of dominant males while non-estrous females showed no preferences (Mossman and Drickamer 1996). Genetic differences in the MHC are conveyed through odors, linking olfactory discrimination and mate choice (Yamazaki and Beauchamp 2007).

Most studies investigating olfactory kin discrimination in the subfamily Xerinae have focused on Holarctic species (Mateo 2003). However, the Cape ground squirrel (*Xerus inauris*), a highly social, semi-fossorial mammal that occurs in southern Africa, displays scent discrimination when exposed to the scents from the feces of predators and non-predators (Belton et al. 2007), as well as venomous and non-venomous snakes (Phillips and Waterman 2013). However, no study has focused on whether or not this species uses odors to discriminate among conspecifics, specifically individuals of the opposite sex during potential mating opportunities.

In the Cape ground squirrel, mating occurs opportunistically throughout the year (Waterman 1998, 2007). Females live in social groups with 1–5 other adult females in addition to 2–3 subadults of either sex (Pettitt and Waterman 2011). Some subadult males disperse and join all-male bands of up 19 individuals, while other males (termed non-dispersed males) delay dispersal from their natal group for a number of years beyond maturation (Waterman 1995). These non-dispersed males participate in the alloparental care of young as well as breeding opportunities with local females (Manjerovic and Waterman 2015). During a short estrus (3 h), females copulate with multiple males, with older males gaining first access, and having more successful copulations than younger males (Waterman 1998; Manjerovic and Waterman 2015). Only 28% of males successfully sire offspring, and after copulation there is no observable difference in the fertilization success of dispersed (usually older) versus non-dispersed (usually younger) males (Manjerovic and Waterman

2015). Non-dispersed males attempt to breed with females from their own social group but related females avoid them, and only one case (of 131 offspring in 30 groups over 5 years) of a non-dispersed male siring an offspring in his own natal group has been documented (Manjerovic and Waterman 2015). Young females often delay maturity in the presence of non-dispersed males from their own social groups, possibly through self-restraint (Jackson et al. 2007; Pettitt and Waterman 2011). This potential inbreeding avoidance may be a result of females discriminating between familiar non-dispersed males (more likely to be closely related to the female) versus dispersed (foreign) males (more likely to be less-closely related) using scent, but as of yet conspecific scent discrimination in this species has not been investigated. We presented pregnant and non-pregnant female Cape ground squirrels with anal gland odors from familiar and foreign males to determine whether female discrimination of familiar and foreign male scent was influenced by female reproductive status, and whether discrimination was based on familiarity and/or correlated with genetic relatedness. Females do not have post-partum estruses and for females that do not give birth (either by miscarrying or failing to conceive) or lose their litters during lactation, interestrous intervals can be long (76 days for the former and 90 days for the latter). Females that breed successfully have even longer interestrous intervals (146 days; Waterman 1996). Thus pregnant females do not seek mates for a considerable time during reproduction (Waterman 1996).

We tested the hypotheses that non-pregnant females would discriminate between the odors of familiar and foreign males, as well as be able to discriminate between the odors of male squirrels that are of varying degrees of relatedness. We predicted that if female squirrels distinguish between the scents of males based on potential mates, (a) non-pregnant females should examine and show a preference towards the odors of foreign males as an indicator of mate choice, to avoid the potential costs of mating with familiar (non-dispersed) relatives; (b) non-pregnant females will discriminate between male conspecifics of varying degrees of relatedness, interacting preferentially with odors from males that are less related; and (c) inbreeding levels would be relatively low in the population. We also tested the hypothesis that pregnant females would not show olfactory discrimination of familiar versus foreign males. We predicted pregnant females would investigate both familiar and foreign scents equally, as mate choice is not a factor for the females during pregnancy.

Methods

Study site

The study was conducted at the S.A. Lombard Nature Reserve located near Bloemhof in the North West Province of South Africa (27°35′S, 25°23′E) from May–July 2014 and May–July 2015, during the arid winter months (Unck et al. 2009; Hillegass et al. 2010). Cape ground squirrels have been studied at this site since 2002. The area of the reserve is 3359 ha (Unck et al. 2009), consisting of uniform short grass savannah on a natural floodplain (Hillegass et al. 2010). Rainfall during this season is less than 10 mm, with the average annual temperature of this region being 17.6 °C, and ranges from − 8.7 °C in the winter to 40 °C in the summer (Herzig-Straschil 1978).

Trapping and handling of squirrels

We trapped 15 burrow clusters that contained at least one adult female and one natal male using Tomahawk live traps (15 × 15 × 50 cm; Tomahawk Live Trap Co., Tomahawk, WI, USA) that were baited with peanut butter and/or bird seeds. We restrained each squirrel trapped in a cloth handling bag to minimize contact and decrease stress (Koprowski 2002; Unck et al. 2009), while they were weighed, sexed, and assessed for their reproductive condition. We used the same handling bag throughout the experiment as it was not feasible to have a unique bag for each squirrel that we handled, despite the possibility of residual chemical cues that may have been transferred between trapping/handling locations (Hare 1994). We determined the reproductive condition of females by nipple status (once females start breeding their nipples swell permanently; Pettitt and Waterman 2011) and palpations for pregnancy, denoting the results as non-pregnant ($p = 0$, day 1–15), at the early stages of pregnancy ($p = 1$, day 15–30), intermediate stages of pregnancy ($p = 2$, day 30–45), or at the final stages of pregnancy ($p = 3$, > 45 days), based on the size of the fetus. We implanted each animal with a passive integrated transponder (PIT tag; AVID Inc., Folsom, LA) that was injected under the skin for permanent identification, and we marked each squirrel with a unique, symmetrical symbol using hair dye (Rodol D; Lowenstein & Sons, Inc., New York, NY, USA), for visual identification at a distance. We collected a small amount of skin from the tip of the tail (1–3 mm) for genetic analysis (see below). All animals were then released at the site of capture.

Scent collection

Greeting behaviors (involving oral scent glands) are frequently observed in same sex squirrel encounters but rarely occur between adult male and female Cape ground squirrels (Waterman 1995). Thus, we chose to use anal gland scents as our odors as both male and female Cape ground squirrels will mark substrate with their anal-genital regions and both sexes will sniff these scents (Straschil 1975). We collected anal gland scents from a non-dispersed (familiar) adult male in a social group and a foreign adult male. We used a foreign male that was either dispersed or originating from a different social group (relative to a focal female) and whose home range was in a different area of the reserve, to reduce the probability that the female had encountered the male before (Scantlebury et al. 2008). During the trapping of a squirrel, we collected scent by rubbing each side of a polyethylene cube (3/4 × 3/4 × 3/4 cm, Acryl Design Ltd. Winnipeg, MB) three times along the anus, while wearing latex gloves to minimize contamination of human scent (Mateo 2006). We used new gloves when handling each scent cube. We placed each inoculated cube in a clean plastic bag, which we stored in a freezer at − 20 °C for up to 24 h (Phillips and Waterman 2013). For each trial, we used an unscented cube as a control. We marked the bags with either the letter L (left), C (center), or R (right) to indicate the scent block's eventual position on the 15 × 50 cm sheet of polypropylene tarp when conducting the trial. We randomized the location of each scent cube on the tarp by drawing a location for each scent from a hat. We performed the randomization process for all trials conducted. We sanitized the cubes (and tarps) before collecting scents by soaking them for 5 min in a 50% vinegar solution, rinsing them in water, and drying them in the sun (Phillips and Waterman 2013).

Behavioral observations

We conducted observations of female squirrels from a blind situated on top of a vehicle or from a viewing tower using 10 × 50 binoculars or 20 × 60 spotting scopes (Bushnell Co., KS, USA), from a minimum distance of 50 m away (Phillips and Waterman 2013). Before each trial, we monitored the average wind speed using a Kestrel 3000 Pocket Weather Meter (Nielsen Kellerman, Chester, PA, USA) over a 1-min period to ensure trials were not performed during high-wind periods (wind speed gusting to over 18 km per hour [kph]), as high winds could affect squirrel behavior and scent dispersal (Fairbanks and Dobson 2007). If the wind speed was above 18 kph, the trial was discontinued until it had fallen below the threshold (Phillips and Waterman 2013). We performed all trials during the course of the day after the squirrels had emerged in the morning (between 0800 h and 1700 h local time).

We conducted scent trials once non-dispersed and foreign scents had been collected and the female was in a trap to ensure the correct focal animal was being observed. We released the female down the closest burrow to the trap and removed the trap from the experimental area. We placed the tarp 30 cm from the burrow entrance, and we placed the inoculated cubes 15 cm away from each other in their correct position as denoted on the bag, so that each block was equidistant from the burrow. We placed 2 ml of peanut butter in front of each cube on the edge of the tarp to attract the squirrel to the experimental setup, and we then moved to the blind and waited for the female squirrel to emerge, whereupon the response of the female to the cubes was recorded. We recorded the preference of the female to the odors from the cube, using a digital audio recorder (8-MB iPod Touch, Apple Inc., California, USA) to record measures of preference as the duration of time (seconds) spent in proximity to each cube (< 5 cm from the cube) and sniffing behavior (the female brought its nose within 5 cm of the cube and the rhinarium and whiskers visibly twitched). We recorded the behavior of the female for 10 min after the first proximity recording, or until she had moved away (> 5 m) from the experimental area. We discarded the trial if the focal female had not emerged from the burrow and approached the experimental setup within 30 min. To minimize observer bias, blinded methods were used when all behavioral data were recorded and analyzed, as we were unaware of which odors were on which cubes during the scent trials.

Microsatellite DNA comparisons

We used tail tissue samples to analyze the coefficient of relatedness (r) and inbreeding coefficients (F) of the squirrels using molecular markers. We extracted the DNA using E.Z.N.A ® Tissue DNA Kits (Omega Bio-tek, Inc., Norcross, GA, USA) and all DNA was amplified using polymerase chain reactions (PCR) using 20 species-specific microsatellite loci developed for this species (Abercrombie et al. 2009; Manjerovic and Waterman 2015). 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). We genotyped the individuals used in trials (hosts or donors) with 92% typed at >15 loci. Although two animals were only genotyped at 11 and 14 loci, respectively, we have found that just 8 loci have adequate polymorphic information content to determine relationships (Manjerovic and Waterman 2015). We used COANCESTRY (v1.0.1.2; Wang 2011) to generate a simulated dataset with known allelic frequencies to determine the best estimator for relatedness. As the triadic likelihood estimator had the highest correlation with the true data, we used it to estimate pairwise relatedness using 10,000 bootstrap permutations in COANCESTRY (Wang 2007, 2011). Coefficient of relatedness values range from zero to one, where zero represents dyads that shared no alleles by common descent, and a value of one represents dyads that shared all alleles by common descent. In addition, we used COANCESTRY to determine inbreeding coefficient values using the triadic likelihood estimator for the population ($n = 310$) of marked Cape ground squirrels at the S.A. Lombard Nature Reserve that had >15 microsatellite loci identified.

Data analysis

We conducted scent trials on 19 adult female squirrels from 15 different social groups. Out of the 19 scent trials we performed, 7 were on pregnant females and 12 were on non-pregnant females. We used a randomized block experimental design, blocking on female identification (ID) to reduce residual variation among females. We performed transformations on our original data set so that the assumptions of a parametric test, including assumptions of a normal distribution and homogeneity of variance, were met. For non-pregnant females, we transformed both sniff duration (square-root transformation) and total duration (log transformation of duration +1). If data did not meet the assumptions after the transformation, we used the equivalent non-parametric test. When performing linear regressions, we checked the residuals to ensure the assumptions were met. All statistical tests were computed using JMP V.10 (SAS Institute Inc., Cary, NC, USA). Data are reported with means ($\bar{x}$) and standard error (SE), and statistical significance was set at $\alpha \leq 0.05$.

We used a two-way ANOVA, blocking on ID, and a Kruskal-Wallis test to test for differences between the time spent (a) sniffing each scent cube and (b) the total time spent at each scent cube (sniffing and proximity measurements) for pregnant and non-pregnant females. As we could not normalize the data for pregnant females, we ran separate tests on pregnant and non-pregnant females. For normalized data, we used a Tukey-Kramer HSD post-hoc test to examine which scent cubes were significantly different, and a Steel-Dwass comparison test for the non-parametric comparison. We used a Wilcoxon test to assess whether there was a significant difference in relatedness between the dyads of female-non-dispersed males compared to female-foreign males. We used linear regressions to test for significant relationships between genetic relatedness and sniffing duration at each cube for pregnant and non-pregnant females. Finally, we graphed the distribution of inbreeding coefficients for the population of marked squirrels to examine the variability in inbreeding coefficients for this population of ground squirrels.

Data availability The datasets generated during and analyzed during the current study are available in the figshare repository, https://doi.org/10.6084/m9.figshare.5566723.v1

Results

Scent trials

We found a significant difference in the duration of sniffing performed during the scent trials of non-pregnant females ($n = 12$) (Fig. 1, two-way ANOVA: $F_{2,\ 22} = 28.04$, $P < 0.0001$). Foreign treatments elicited more sniffing behavior than the control (Tukey-Kramer post-hoc test: $P < 0.0001$) and familiar ($P = 0.0125$) treatments. As well, familiar treatments elicited more sniffing than control treatments ($P = 0.0008$). We also found differences in the total duration of time spent at each treatment (Fig. 2, $F_{2,\ 22} = 13.65$, $P = 0.0001$). Non-pregnant females spent a longer duration at foreign and familiar treatments than control treatments ($P = 0.0001$, $P = 0.0047$, respectively). However, there was no difference in the total duration spent at the foreign and familiar treatments ($P = 0.30$).While we found no significant difference in the duration of sniffing (Fig. 1, Kruskal-Wallis: $\chi^2_2 = 1.70$, $P = 0.43$) for scent trials of pregnant females ($n = 7$), we did find a significant difference in total duration (Fig. 2, $\chi^2_2 = 6.64$, $P = 0.04$). However, no pairs were significantly different from one another (Steel-Dwass: all $P \geq 0.08$). While the sniff and total duration results for non-pregnant females had high power (0.60 and 0.58, respectively), the sniff duration and total duration results of pregnant females had a low power (0.05 and 0.054, respectively), because of our small sample size. However, we would need a sample size of greater than 1000 to achieve adequate statistical power (0.5).

Microsatellite DNA analysis

We found a significant difference between the relatedness values of non-pregnant females ($n = 10$) to the familiar versus foreign males used in the scent trials (Fig. 3, Wilcoxon test: $\chi^2_1 = 11.57$, $P = 0.0007$) and familiar and foreign males in the pregnant scent trials (n = 7) (Fig. 3, $\chi^2_1 = 4.44$, $P = 0.04$). We found no significant relationship between genetic relatedness and sniff duration for non-pregnant females (familiar male scent: $F_{1,\ 9} = 0.17$, $P = 0.69$; foreign male scent: $F_{1,\ 9} = 0.02$, $P = 0.88$) or pregnant females (familiar male scent: $F_{1,\ 6} = 1.38$, $P = 0.29$; foreign male scent: $F_{1,\ 5} = 1.06$, $P = 0.36$).

We computed the inbreeding coefficients for all marked Cape ground squirrels, including both males and females

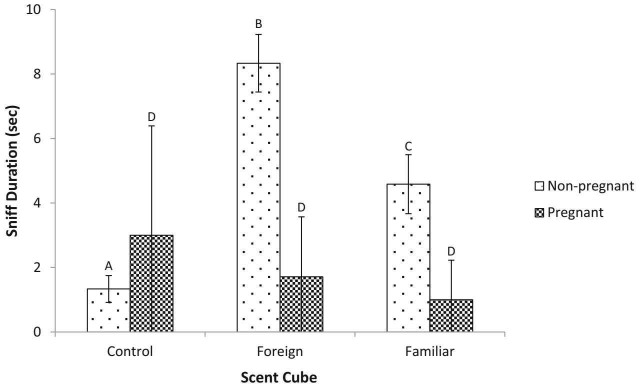

Fig. 1 The duration (seconds) spent sniffing in non-pregnant versus pregnant female Cape ground squirrels in response to different odors (control, foreign, and natal males; $n = 12$ for non-pregnant scent trials, $n = 7$ for pregnant scent trials). Mean values (± SE) are shown for each of the three treatments. Treatments not connected by the same letter are significantly different ($P < 0.05$; A–C for non-pregnant scent trials, D–F for pregnant scent trials)

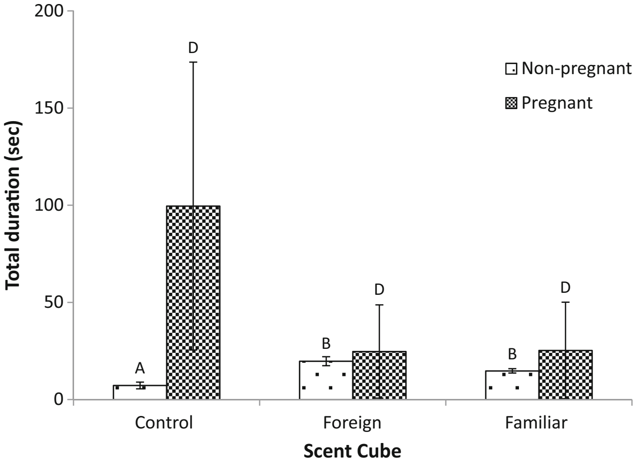

Fig. 2 The total duration (sniff and proximity) (seconds) in non-pregnant and pregnant female Cape ground squirrels spent at different odors (control, foreign, and non-dispersed males; $n = 12$ for non-pregnant scent trials, $n = 7$ for pregnant scent trials). Mean values (±SE) are shown for each of the three treatments. Treatments not connected by the same letter are significantly different ($P < 0.05$; A–C for non-pregnant scent trials, D–F for pregnant scent trials)

($n = 310$). The majority of the squirrels in the population had low inbreeding coefficients (Fig. 4), with 68% of the population having an inbreeding coefficient less than 0.10.

Discussion

Non-pregnant female Cape ground squirrels discriminate between the scents of familiar and unfamiliar male conspecifics, spending significantly longer sniffing the foreign male scent than either the familiar or control scent cubes. Pregnant females do not appear to display the same level of olfactory discrimination of males as non-pregnant females. While we found a significant difference between the relatedness coefficients of female-familiar dyads and female-foreign male dyads for both pregnant and non-pregnant females, there was no significant relationship between the relatedness of test individuals and the duration of time spent sniffing the scent cubes. As well, when we examined the marked Cape ground squirrel population, we found that the majority of individuals (both males and females) had low inbreeding coefficients.

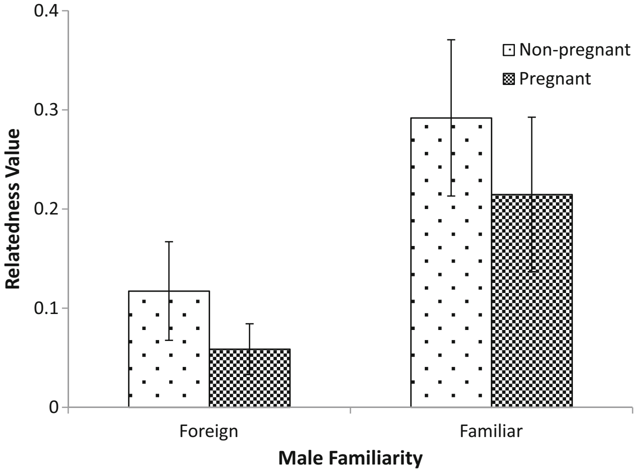

Fig. 3 Comparing the coefficient of relatedness (r) values (mean±SE) of the focal non-pregnant and pregnant females to the foreign males and non-dispersed males from the Cape ground squirrel population used in each scent trial ($n = 10$ for non-pregnant females, $n = 7$ for pregnant females)

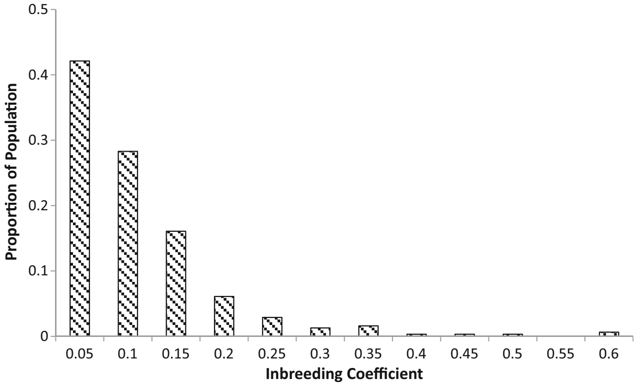

Fig. 4 The proportion of inbreeding coefficients (F) for the marked population of Cape ground squirrels ($n = 310$) using a bin width of 0.05

Our results are consistent with studies performed on other rodents, including the naked mole-rat (*Heterocephalus glaber*), where reproductively active females consistently preferred odors from unfamiliar males over familiar males and reproductively inactive females did not discriminate between foreign and familiar males (Clarke and Faulkes 1999). Tuco-tucos (*Ctenomys talarum*), meadow voles (*Microtus pennsylvanicus*), and common degus (*Octodon degus*) also display olfactory discrimination of conspecifics on the basis of familiarity (Ferkin 1988; Zenuto and Sol Fanjul 2002; Villavicencio et al. 2009).

Discrimination of conspecifics on the basis of familiarity can prove beneficial in terms of the *dear enemy* effect, as well as kin and mate selection. The *dear enemy* effect is observed in territorial species where individuals display reduced aggression towards familiar conspecifics with neighboring territories, while maintaining aggression towards unfamiliar conspecifics (Fisher 1954; Rosell and Bjørkøyli 2002). Evidence for the *dear enemy* effect has been documented in taxa ranging from amphibians (Lesbarrères and Lodé 2002) to birds (Brindley 1991), where auditory signals are the primary mechanism used for recognition. The *dear enemy* effect has also been observed in rodents, through the use of olfactory cues (Ferkin 1988; Murdock and Randall 2001). However, in the Cape ground squirrel, neither males nor females are territorial, and furthermore, agonistic encounters between conspecifics are rarely observed (Waterman 1995).

As the *dear enemy* effect does not appear to apply to Cape ground squirrels, olfactory discrimination based on familiarity may have evolved in the context of kin or mate selection. Discrimination of odors that convey information about familiarity and genetic relatedness may optimize the balance between inbreeding and outbreeding (Bonadonna and Sanz-Aguilar 2012; Leclaire et al. 2013). There is the potential for a high level of inbreeding in this population of Cape ground squirrels, as the highest relatedness value for a female-familiar male dyad in the scent trial experiments was $r = 0.74$. However, when examining the range of inbreeding coefficients in this population, low to moderate levels of inbreeding were generally observed. High levels of inbreeding can be common in small populations of animals, as was observed in a population of gray wolves (*Canis lupus*), where the majority of individuals born after 1997 had inbreeding coefficients close to 0.25 (Liberg et al. 2005). Suricates (*Suricata suricatta*) display similar levels of low to moderate inbreeding as the Cape ground squirrel, and high levels of inbreeding affected the viability and success of a number of different traits (Nielson et al. 2012). However, female suricates spend a longer time investigating the scent of unfamiliar males only if they were closely related (Leclaire et al. 2013). In banded mongoose (*Mungos mungo*), both sexes display kin discrimination as a means of reducing the occurrence of inbreeding, and females appear to choose mates that are more distantly related (Sanderson et al. 2015). The variation in responses of pregnant and non-pregnant females to the odors of non-dispersed (familiar) and foreign males indicates that scent discrimination may be involved in mate selection in Cape ground squirrels. Furthermore, the low inbreeding coefficients for this population of squirrels suggests that females may discriminate among males based on relatedness, as close inbreeding between relatives appears to be avoided (Manjerovic and Waterman 2015). While our results suggest olfactory discrimination plays a role in mate choice, there may still be benefits to identifying kin that were not investigated in this experiment.

Familiarity is a fairly reliable indicator of relatedness in the Cape ground squirrel in the context of mate choice. The social structure of this species does result in the mixing of kin and non-kin during mating events that occur year-round, when males attempt to mate with females from different burrow clusters (Waterman 1998). These mating events contrast with the naked mole-rat, where individuals occupy discrete burrow systems, and mixing of kin and non-kin is relatively rare (Jarvis et al. 1994). However, females and non-dispersed male Cape ground squirrels live in discrete burrow systems separate from dispersed males, and non-dispersed males that do disperse generally leave the area of their natal burrow cluster (Waterman 1995). Therefore, unfamiliar males that are encountered by females are more likely to be less related or unrelated compared to familiar, non-dispersed males. The dispersal of one sex is a widely accepted mechanism promoting inbreeding avoidance in animals, as the coancestry of potential mates is reduced (Bengtsson 1978; Waser et al. 1986). In addition to familiarity-based recognition cues, other post-copulatory mechanisms that may help to reduce inbreeding in this population include cryptic female choice (Bretman et al. 2009; Løvlie et al. 2013), the decreased fitness of inbred offspring (Ralls and Ballou 1982; Acevedo-Whitehouse et al. 2003), and reproductive delay of females (Jackson et al. 2007; Pettitt and Waterman 2011). Other factors that we did not examine that may influence female preference of male odors include the level of parasitism (Møller et al. 1990; Kavaliers and Colwell 1995; Patterson and Schulte-Hostedde 2011) and diet (Beauchamp 1976; Ferkin et al. 1997) of males. If only the novelty of the odor was the main stimulus driving scent discrimination by females (Holmes 1984; Zenuto and Sol Fanjul 2002) we would expect to observe similar responses in pregnant and non-pregnant females, which did not occur.

When taking into account both proximity and sniffing duration together, there was no significant difference between the total duration non-pregnant females spent at the foreign male scent versus the non-dispersed male scent. However, the total duration of time spent at the non-dispersed and foreign cubes was significantly greater than the unscented control. Since we placed bait equally in front of all scent cubes during the experiment, we would expect no difference in the total duration spent at any of the cubes if females displayed no olfactory discrimination. Since females spent less time at the control cube than the non-dispersed and foreign male scents, this result suggests further evidence that females do show an olfactory preference. However, proximity may be a less powerful predictor of discrimination than sniffing behavior, since sniffing is often observed as a method of mate inspection, using odors produced from scent glands (Barnard et al. 1991; Mateo 2006).

In conclusion, there is evidence that non-pregnant female Cape ground squirrels discriminate between the odors of foreign and familiar males. Olfactory discrimination may be used, in addition to other mechanisms, to maintain low levels of inbreeding within the population, as pregnant females who are not actively seeking males displayed no preference to the odors of familiar or foreign males. Further experimental and molecular studies should be directed towards examining the impact of inbreeding depression on this population, as well as the use of other odors, such as oral and urinary scents (Mateo 2006), as a method used for social communication among conspecifics in the Cape ground squirrel.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures were made in accordance with the American Mammal Association guidelines (Sikes and Gannon 2011), and were approved by the University of Manitoba's Animal Care and Use Committee (#F14-032).

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