Kinship, ectoparasites, and reproductive success in Cape ground squirrel (*Xerus inauris*) social networks

by

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Abstract

Sociality – or living in groups among members of the same species – can have both costs and benefits for survival and reproductive success. Inclusive fitness theory suggests organisms might behaviourally favour closer kin if they can identify and act to benefit them. In promiscuous social species, average relatedness may be too low to maintain costly social behaviours like cooperative breeding without kin discrimination. Animals that are more central in social networks may gain fitness benefits and costs similar to those of sociality generally, but particularly those effects that arise from indirect social connections. Increased social network centrality is often linked to improved fitness, but also greater ectoparasite abundance. Exploring whether fitness correlates to social network position under low rates of aggression or hierarchy remains a key challenge for comparative evolutionary ecology. Cape ground squirrels (*Xerus inauris*) are facultatively cooperative breeders with high promiscuity. They are a social species that rarely exhibit aggressive behaviours towards conspecifics and show no evidence of dominance hierarchies. A previous study that removed their parasites dramatically increased female reproductive success. Our objective was to examine whether kinship influences social structure and whether ectoparasite abundance and centrality influence survival and reproductive success in adult female Cape ground squirrels. We predicted kinship increases affiliative and decreases agonistic interactions, that centrality increases ectoparasites, and that ectoparasites and centrality reduce reproductive success but not survival. We studied Cape ground squirrels on S.A. Lombard Nature Reserve, South Africa in 2017. We followed 14 social groups, collected interaction data through all-occurrence sampling, and collected ectoparasite abundance data from trapping. We found that kinship increased both affiliative and agonistic interactions. Since agonistic interactions are relatively mild in this species, greater agonistic interaction frequencies with closer kin may have insignificant fitness costs. More central adult females had more ectoparasites, but eigenvector centrality and ectoparasite abundance did not affect survival or reproductive success. Our study shows that Cape ground squirrels are nepotistic with their interactions within their social groups, and that the costs and benefits of group living in species with low rates of aggression can have novel implications for the relationships between fitness and sociality.

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Chapter 1: Thesis introduction

Sociality – or living in groups among members of the same species – can have both costs and benefits for survival and reproductive success (Alexander 1974; Silk 2007; Clutton-Brock 2009; Smith 2014). Sociality has evolved in all major mammalian lineages and across broad ecological and evolutionary contexts, suggesting strong selection for this behaviour (Smith et al. 2017). Living alongside conspecifics can increase competition for resources and reproductive opportunities, attract predator attention, and expedite disease and parasite transmission, among other costs (Kutsukake & Clutton-Brock 2006; Treisman 1975; Silk et al. 2018; Wey & Blumstein 2012). The fitness benefits of sociality may include enhanced predator avoidance, improved mate access, communal habitat alteration, thermal conservation, reciprocal parasite removal, cooperative breeding, and communal territorial defense, among other benefits (van der Marel 2019; Altizer et al. 2003; Johnson et al. 2004; Garroway et al. 2013; Mosser & Packer 2009; Pulliam 1973; Treisman 1975; Bertram 1978; Hamilton 1971). Thus, conspecifics must navigate between the positive and negative effects of groupmates to maximize their fitness.

Survival and reproductive benefits also may arise via mutualism, reciprocity, or group augmentation (Clutton-Brock 2009; Kingma et al. 2014). Individuals may also mitigate the costs or increase the benefits of sociality via kin selection (Queller 2016). Kin selection theory predicts nepotistic behaviour – the favouring of close kin in social interactions – as a proposed explanation for the evolution of apparently altruistic cooperation due to the indirect fitness benefits of helping individuals with a high proportion of shared alleles (Hamilton 1964; Maynard Smith 1964).

Proximately, kin selection requires an ability to determine – or a generally applicable behavioural strategy to reliably approximate – the degree of relatedness between interactors (Mateo 2002; Smith 2014). Kin discrimination can be accomplished via phenotype matching or familiarity (Tang-Martinez 2001). Several animal taxa can discriminate relatedness phenotypically, often through olfactory cues, with many examples in rodents, carnivores, and primates (Mateo 2002; see references in Smith 2014). Golden-mantled ground squirrels (*Callospermophilus lateralis*) recognize kin but do not discriminate to treat their closer kin preferentially (Mateo 2002). Therefore, golden-mantled ground squirrels demonstrate that the presence of kin recognition in a species does not necessarily imply the presence of nepotism (Mateo 2002).

Inclusive fitness theory suggests organisms might behaviourally favour closer kin if they can identify highly related kin and act to benefit them, and if the benefits of doing so outweigh the costs to themselves at a likelihood weighted by the degree of relatedness between the interactors (Hamilton 1964). In promiscuous social species, average relatedness may be too low to maintain costly social behaviours like cooperative breeding – where individuals forego reproduction to help others reproduce, often delaying their sexual maturity or dispersal (Cornwallis et al. 2010; Kramer & Russell 2014; Blumstein & Armitage 1999). Monogamy has been suggested to be a prerequisite for cooperative breeding, since monogamy keeps social group relatedness high, which increases the inclusive fitness benefits of helping group members (Cornwallis et al. 2010; Kramer & Russell 2014). This 'monogamy hypothesis' does not explain cooperative breeding observed in promiscuous species, except if the species is able to discriminate kin (Cornwallis et al. 2010; Kramer & Russell 2014). If, due to promiscuous breeding behaviour, genetic variance is high within a social group, then the chances are high that even familiar animals will have low relatedness with each other (Mateo 2002; Queller 2016). For individuals in promiscuous species to gain inclusive fitness benefits through nepotistic behaviours, they require kin discrimination on a finer degree of relatedness beyond just a principle of recognizing familiarity (Mateo 2002; Queller 2016). Therefore, in promiscuous species, the evolution of cooperative breeding may require kin recognition and nepotistic behaviour to allow individuals to favour closer kin when average group relatedness is low (Cornwallis et al. 2010; Kramer & Russell 2014).

Intragroup social interactions are considered one of the fundamental components determining social complexity (Kappeler 2019). Traditional analyses of paired (dyadic) social interactions are informative but insufficient to characterize the structure of complex social groups (Makagon et al. 2012; Krause et al. 2015). Social network analysis allows consideration of indirect social connections (Newman 2003; Krause et al. 2015). Centrality measurements are one class of network metric that quantify the relative importance of an individual within a network of social interactions, allowing social position to be quantitatively compared to other individual variables, such as fitness measures (Newman 2003; Krause et al. 2015). Eigenvector centrality measures the interaction degree (the number of interactors) of an individual weighted by the degree of those interactors in turn (Newman 2003; Bonacich 2007; Makagon et al. 2012; Cheney et al. 2016). Social network position may have evolutionarily important fitness

consequences when a relationship exists between centrality and reproductive success (Brent 2015). More favourable network positions correlate positively to individual longevity and fitness estimates in a variety of animal taxa (Formica et al. 2012; Farine & Sheldon 2015; Silk et al. 2010; Cheney et al. 2016; Schülke et al. 2010; Cameron et al. 2009; Natoli et al. 2007; von Holst et al. 2002; Yee et al. 2008; Wey & Blumstein 2012; Stanton & Mann 2012). Animals that are more central in social networks may gain fitness benefits and fitness costs that are similar to the benefits and costs of social living generally, but particularly those benefits and costs that arise from indirect social connections (Makagon et al. 2012; Brent 2015).

However, high rates of direct and indirect interactions can also be costly (Krause et al. 2015; Evans et al. 2020). One cost of high interactions is the potential to transmit infections and parasites (Godfrey 2013; Lucatelli et al. 2020). For example, social structure positively associates with parasitism in eastern chipmunks (*Tamias striatus*), Natterer's bats (*Myotis nattereri*), meerkats (*Suricata suricatta*), and Tana River red colobus monkeys (*Piliocolobus rufomitratus*) (Lucatelli et al. 2020). In animal groups, increased social network centrality is often linked to improved fitness, but also to greater parasite abundance (Brent 2015; Godfrey 2013). Parasites vary in the significance of their fitness costs, but the costs can become severe (Patterson et al. 2013; Akinyi et al. 2019). If costly parasites pass between animals, high rates of interaction between more connected (central) individuals should be more costly than interactions with less central individuals (Evans et al. 2020; Silk et al. 2018). As social group size increases, the total parasites and parasites per individual may increase (Hoogland 1979; Brown & Brown 2004; Rifkin et al. 2012). Larger group sizes can increase parasite abundance if larger groups experience more interactions among conspecifics, or by increasing host density and thus availability for parasites, or if larger groups of animals are more noticeable to mobile parasites or their vectors (Dunbar 1991; Rifkin et al. 2012; Nunn & Heymann 2005). Animals may mitigate the increasing abundance of ectoparasites (parasites that infect the outside of their host) in larger social groups by removing ectoparasites from each other, termed allogrooming (the grooming of other individuals), a strongly affiliative behaviour (Kutsukake & Clutton-Brock 2006).

If ectoparasites are spread via social contact, it follows that greater social contact may increase an individual’s ectoparasite abundance (Altizer et al. 2003). In a recently published meta-analysis of the relationship between sociality and parasitism in mammals, parasitism is predicted by social interactions (Lucatelli et al. 2020). Furthermore, greater social contact with

individuals who themselves have greater social contact is likely to increase an individual's ectoparasite abundance even more so (Brent 2015). The summed weight of such indirect social connections can be measured with the social network metric termed eigenvector centrality (Bonacich 2007; Cheney et al. 2016). More central individuals have higher parasite abundances in a variety of taxa, including reptiles (Fenner et al. 2011; Leu et al. 2010; Godfrey et al. 2010) and mammals (MacIntosh et al. 2012, but see Duboscq et al. 2016; Rimbach et al. 2015; VanderWaal et al. 2014; Corner et al. 2003). Ectoparasites are also often more likely to infect individuals who physically contact others, individuals who allogroom, and individuals who associate with infected individuals (MacIntosh et al. 2012; Rimbach et al. 2015; White et al. 2017). In the above-mentioned meta-analysis of the relationship between sociality and parasitism in mammals, parasitism is predicted by social interactions but not by group size (Lucatelli et al. 2020). The effects of group size and social structure on parasitism did not depend on parasite type (endoparasites or ectoparasites), nor on the metric used to measure the parasites (richness, abundance, or prevalence), nor on whether parasitism was measured at the scale of the individual or at the scale of the group (Lucatelli et al. 2020). Lucatelli et al. (2020) join Kappeler (2019) in advocating for more complete descriptions of species' social system complexity to compare a full suite of social factors to fitness consequences like those potentially accrued from parasitism. Group size alone is an insufficient measurement of the degree of sociality, because, for example, small groups can have frequent interactions, and large groups can have infrequent interactions (Lucatelli *et al*. 2020).

In this thesis, we use Cape ground squirrels (*Xerus inauris*) as a study system to investigate the topics of nepotism, sociality, and fitness consequences via parasitism in a highly social, sexually promiscuous, and cooperatively-breeding, ground-dwelling rodent (Waterman 1995, 1996). Cape ground squirrels investigate odours from closer kin longer when presented with scents from unfamiliar squirrels, but not when presented with scents from familiar squirrels (Waterman & Archibald 2019). So, Cape ground squirrels can use phenotype matching from olfactory cues to detect relatedness to a fine degree, but they do not appear to discriminate the scents of squirrels from their social groups (Waterman & Archibald 2019). Whether Cape ground squirrels use phenotype matching to treat kin preferentially in social interactions within their social groups has not yet been investigated (Waterman & Archibald 2019).

In addition, a previous study at our same study site found that ectoparasites did not increase with group size in Cape ground squirrels (Hillegass et al. 2008). Experimentally removing parasites greatly increased reproductive success among adult females (Hillegass et al. 2010). Experimental removal of ectoparasites almost eliminated both autogrooming and allogrooming behaviour in Cape ground squirrels, indicating their grooming behaviour is stimulus-driven by the presence of ectoparasites (Hillegass et al. 2010). Female Cape ground squirrels lack dominance hierarchies, so their allogrooming behaviour does not serve to improve their hierarchic social positions, a typical role of allogrooming in primates (Waterman 1995; Schino 2001). Whether Cape ground squirrel allogrooming frequencies correlate to ectoparasite abundances has not yet been investigated.

Social systems are characterized by how social groups are organized (i.e. how big their groups are; do their group compositions depend on sex, age, or kinship), how they are structured (i.e. who interacts with whom within the group, or between groups), their mating systems, and their juvenile care systems (Kappeler 2019). The first part (Chapter 2) of this thesis explores the complexity of Cape ground squirrel social groups at the level of the dyad, or pair-wise relationships. Our dyadic variables include pair-wise relatedness, interaction frequencies, and whether interaction frequencies are biased by homophily: specifically, if the interactors match each other in terms of sex, age class, or social group. We also examine whether the average pair-wise relatedness within social groups is greater than expected by chance. The second part of this thesis (Chapter 3) explores Cape ground squirrel social complexity at the level of the individual. Our individual-level variables include interaction frequencies, allogrooming frequencies, centrality within social interaction networks, and how well those social interaction variables predict individual ectoparasite abundance and estimates of fitness. We also analyze the effects of group size on allogrooming rates and ectoparasite abundance.

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Chapter 2: Kinship and social network structure in Cape ground squirrels (*Xerus inauris*)

Abstract

Monogamy has been suggested as a prerequisite for the evolution of cooperative breeding, since monogamy keeps social group relatedness high, which increases the inclusive fitness benefits of helping by group members. This 'monogamy hypothesis' does not explain cooperative breeding observed in promiscuous species, although an exception arises when social group members can discriminate their behaviour based on kinship. Few examples exist of cooperative breeders that are also promiscuous. Cape ground squirrels (*Xerus inauris*) are facultatively cooperative breeders with high promiscuity. They can recognize their degree of relatedness with other squirrels by scent but they did not discriminate sniffing duration among familiar squirrels, suggesting they may not discriminate kinship amongst their social group members. Our objective was to examine whether kinship influences the within-group social structure of Cape ground squirrels. We hypothesized that Cape ground squirrels exhibit behavioural kin biases due to inclusive fitness benefits and interact affiliatively more often and agonistically less often with closer kin within their social groups. We also predicted relatedness would correlate to interaction frequencies even among non-offspring-parent pairs. We studied Cape ground squirrels on S.A. Lombard Nature Reserve, South Africa in the austral winter of 2017. We followed 14 social groups and collected interaction data through all-occurrence sampling. We found that Cape ground squirrels biased their interaction networks to interact more often with closer kin within their social groups both affiliatively and agonistically. Cape ground squirrels were also more affiliative with group members of their same sex and more affiliative with group members of a different age class (adult vs subadult). Sex biases may be due to direct and indirect fitness benefits associated with predator avoidance and natal philopatry, and age biases may be due to direct fitness benefits associated with pay-to-stay alloparental behaviour. Relatedness was correlated with interaction frequencies beyond just parental care. Our study shows that Cape ground squirrels are nepotistic with their interactions within their social groups; however, the fitness consequences of their interactions remain unknown.

Introduction

Sociality – or living in groups among members of the same species – can have both costs and benefits for survival and reproductive success (Alexander 1974; Silk 2007; Clutton-Brock 2009; Smith 2014). Some of the costs of sociality include increased resource and mate competition, attracting predator attention, increased transmission of parasites and diseases, and greater risk of inbreeding (Alexander 1974; Silk 2007; Clutton-Brock 2009; Schoepf & Schradin 2012). Some of the benefits of sociality include predator detection, predation dilution, predation defense, parasite removal, thermal conservation, habitat improvement, and mate access (Hare 2004; Clutton-Brock 2009; Covas & Doutrelant 2019). Survival and reproductive benefits also may arise via mutualism, reciprocity, or group augmentation (Clutton-Brock 2009; Kingma et al. 2014). Individuals may also mitigate the costs or increase the benefits of sociality via kin selection (Queller 2016). Kin selection theory predicts nepotistic behaviour – the favouring of close kin in social interactions – as a proposed explanation for the evolution of apparently altruistic cooperation due to the indirect fitness benefits of helping individuals with a high proportion of shared alleles (Hamilton 1964; Maynard Smith 1964).

Proximately, kin selection requires an ability to determine or a generally applicable behavioural strategy to reliably approximate the degree of relatedness between interactors (Mateo 2002; Smith 2014). Kin discrimination can be accomplished via familiarity or phenotype matching (Tang-Martinez 2001). Several animal taxa can discriminate relatedness phenotypically, often through olfactory cues, with many examples in rodents, carnivores, and primates (Mateo 2002; see references in Smith 2014).

Inclusive fitness theory suggests organisms might behaviourally favour closer kin if they can identify highly related kin and act to benefit them and if the benefits of doing so outweigh the costs to themselves at a likelihood weighted by the degree of relatedness between the interactors (Hamilton 1964). In promiscuous social species, average relatedness may be too low to maintain costly social behaviours like cooperative breeding – where individuals forego reproduction to help others reproduce, often delaying their sexual maturity or dispersal (Cornwallis et al. 2010; Kramer & Russell 2014; Blumstein & Armitage 1999). Monogamy has been suggested to be a prerequisite for cooperative breeding, since monogamy keeps social group relatedness high, which increases the inclusive fitness benefits of helping group members (Cornwallis et al. 2010; Kramer & Russell 2014). This ‘monogamy hypothesis’ does not explain

cooperative breeding observed in promiscuous species, except if the species is able to discriminate kin (Cornwallis et al. 2010; Kramer & Russell 2014). If, due to promiscuous breeding behaviour, genetic variance is high within a social group, then the chances are high that even familiar animals will have low relatedness with each other (Mateo 2002; Queller 2016). For individuals in promiscuous species to gain inclusive fitness benefits through nepotistic behaviours, they require kin discrimination on a finer degree of relatedness beyond just a principle of recognizing familiarity (Mateo 2002; Queller 2016). Therefore, in promiscuous species, the evolution of cooperative breeding may require kin recognition and nepotistic behaviour to allow individuals to favour closer kin even when average group relatedness is low (Cornwallis et al. 2010; Kramer & Russell 2014). Few examples exist of cooperative breeders that are also promiscuous. Superb fairy-wrens (*Malurus cyaneus*) and Australian magpies (*Gymnorhina tibicen*) are cooperative breeders and highly promiscuous (Mulder et al. 1994; Finn & Hughes 2001). Australian magpies did not bias their alloparental care – i.e. the care of juveniles other than their offspring – in favour of closer kin, as no difference was found among helping behaviour rates relative to recipient relatedness (Finn & Hughes 2001). Superb fairy-wrens were hypothesized to direct paternal care only towards their offspring, but a DNA analysis found 76% of offspring were raised by a male that was not their father (Mulder et al. 1994).

Cape ground squirrels (*Xerus inauris*) are facultative cooperative breeders with high promiscuity (Waterman 1998; Pettitt & Waterman 2011; Manjerovic & Waterman 2015). They are a highly tolerant species with frequent allogrooming and other affiliative behaviours and few aggressive interactions (Waterman 1995; Pettitt & Waterman 2011; van der Marel 2020). Cape ground squirrels are not territorial, and females live in matrilineal social groups composed of 1–5 related females and their offspring (Waterman 1995; Hillegass et al. 2008). Both sexes mate multiply, and even though litter size is small (1-2 offspring), nearly 70% of litters of two are multiply sired (Waterman 1995, 1998; Manjerovic & Waterman 2015). Adult males who are still living in their family group often provide alloparental care – the care of non-descendant offspring – to offspring in their social group, and their rates of care are not correlated with kinship (LaFlèche et al. *Submitted*). Relatedness within Cape ground squirrel social groups can vary widely (Manjerovic & Waterman 2015; Shave & Waterman 2017). Their high variability of relatedness within groups makes it possible to measure kinship and test its relationship to other

pair-wise variables, like interaction frequency and whether interactors match sex or age (Smith 2014).

It follows from the kin selection hypothesis for cooperative breeding that Cape ground squirrels should have kin-biased interactions (Cornwallis et al. 2010; Kramer & Russell 2014). Waterman & Archibald (2019) found that Cape ground squirrels can recognize their degree of relatedness with other squirrels by scent alone but that they did not discriminate among familiar squirrels, suggesting they may not discriminate kinship amongst their social group (Waterman & Archibald 2019). The ability to recognize kinship phenotypically suggests that the squirrels could interact with kin preferentially, but it is unclear whether they do (Waterman & Archibald 2019).

The objective of our study was to examine whether kinship influences the social structure of Cape ground squirrels. We hypothesized that Cape ground squirrels would exhibit behavioural kin biases due to inclusive fitness benefits (Hamilton 1964). This hypothesis predicts that Cape ground squirrels will interact affiliatively more often and agonistically less often with closer kin within their social group (Silk 2009; Van Belle et al. 2014; Viblanc et al. 2016). We also hypothesized that if kin selection is an important evolutionary mechanism in this species' social interactions, we will see relatedness is correlated with interaction frequencies beyond just parental care, since parental care arises from direct fitness (Hamilton 1964). This hypothesis predicts that we will detect relatedness correlating to interaction frequencies even among non-offspring-parent pairs.

We used social network analyses to understand more about Cape ground squirrel social behaviour and social systems: specifically, by building weighted networks of affiliative and agonistic interaction events among social groups observed in the field and testing whether the edge weights of those networks are correlated to kinship. Social network analyses have not been previously examined in Cape ground squirrels.

Methods

Study site

We collected data from April to September 2017 in central South Africa at S.A. Lombard Nature Reserve (3660 ha, 18 km northwest of Bloemhof, North West Province, 27º35'S, 25º23'E), an arid grassy floodplain ecosystem dominated by tall perennial grasses such as

turpentine grass (*Cymbopogon plurinodis*) and red grass (*Themeda triandra*; van Zyl 1965). Cape ground squirrels have been studied at this site since 2002 (Hillegass et al. 2008; Pettitt & Waterman 2011). Temperatures ranged from -3.5 ºC at night to 27.8 ºC during the day. The site has an average annual rainfall of 502 mm with a range of 241–965 mm from the years 1952–2004, most of which occurs in the months November to April (Pettitt and Waterman 2011; Herzig-Straschil 1978).

Biology of the study animal

Cape ground squirrel social groups lack detectable dominance hierarchies, and all individuals in a group share a communal feeding range (Herzig-Straschil 1978; Waterman 1995; Pettitt & Waterman 2011). Females remain in the social group they are born in – termed matrilineal philopatry (Waterman 2002). We define a social group as related female squirrels and their offspring that are observed to sleep together in a single burrow cluster (Waterman 1995). Social groups dig connected groups of burrows in distinct clusters isolated from other clusters (Waterman 1995). Males either disperse at maturity or delay dispersal for months or up to 5 years (hereafter termed natal males; Manjerovic & Waterman 2015; O'Brien et al. *In Press*). After males disperse from their natal burrow clusters (hereafter termed band males), they join all-male bands that live independently of female social groups (Waterman 1995, 1996, 1998). Social groups have mostly permanent stable residences in one burrow cluster, while band males form ephemeral 'fission-fusion' groups that are transient among burrow clusters throughout the floodplain (Scantlebury et al. 2008). Both natal and band males search the floodplain for females in estrus (Waterman 1998; Manjerovic & Waterman 2015). Females enter estrus year-round and spontaneously (Bouchie et al. 2006) and mate with multiple males at an operational sex ratio of eleven males to one female (Waterman 1998). During a short (3 hours) estrus, females mate with an average of four males (Waterman 1998).

Trapping and measurements

We trapped squirrels with Tomahawk live traps (Tomahawk Live Trap Inc., WI; 15 X 15 X 50 cm) baited with peanut butter mixed with crushed bird seed. We then transferred the squirrels to cone-shaped handling bags (Koprowski 2002) that allowed us to record body measurements and reproductive condition. We considered females to be adults when they started breeding, indicated by nipples that were visibly swollen (nipples swell permanently at first

estrus; Pettitt & Waterman 2011) or extended from lactation, or if their vulva was swollen, which indicates estrus (Waterman 1996; Pettitt et al. 2008). We determined males were adults if their testes had descended into their external scrotum (Waterman 1996). Immature squirrels do not have swollen nipples or descended testicles (Waterman 1996). We classified squirrels without swollen nipples or descended testicles as subadults (Waterman 1995; Pettitt et al. 2008). We measured body mass (± 5.0 g) using a spring scale (Pesola AG, Baar, Switzerland). We also injected each squirrel with a passive integrated transponder (PIT tag; AVID Inc., Norco, CA, U.S.A.) for permanent unique identification, created a small freeze mark (Lectro-Freeze Communica Ltd., Pretoria, South Africa; Rood & Nellis 1980) for permanent identification at a distance, and painted a large unique dye mark (Rodol D, Lowenstein and Sons Inc., New York, NY, U.S.A.) for temporary individual identification at a distance. We also collected a small amount of skin (3mm) from the tip of the tail for genetic analysis (see below). We then released the squirrels at their capture site. Our methods followed the 2016 Guidelines of the American Society of Mammalogists (Sikes 2016). The University of Manitoba's Animal Care and Use Committee (F14-032) approved all research protocols.

Behavioural observations

We recorded squirrel behaviours using scan (every 10 minutes) sampling to record the activities and estimated locations of all visible squirrels and by recording all social interactions using an Android application designed for this project (all-occurrences sampling; Altmann 1974). We used 10 x 50mm binoculars and 15-45 x 60mm spotting scopes (Spacemaster, Bushnell Co. KS, U.S.A.) and concealed ourselves atop towers and vehicle hides. We focused our observation sessions on one or two focal social groups selected with a random number generator per session but recorded all squirrels visible to us. We defined social interactions by an ethogram in Waterman (1995) that includes grooming another squirrel (allogrooming), touching snouts (greeting), chasing, following, jumping back, and jumping in the air past each other (ritualized fighting). We did not include days of estrus in our observations, because males and females behave differently on those days (Waterman 1998) and we excluded band males from our analyses as they do not live with the females (Waterman 1995, 1997).

We divided social behaviours into affiliative interactions ('socially cohesive'), and agonistic interactions ('socially competitive'; Wey & Blumstein 2010). We considered affiliative interactions as those social interactions with the apparent intent of increasing or maintaining

social cohesion among squirrels, including 'allogrooming,' 'greeting', approaching, playing, following, and rolling over to solicit allogrooming ('presenting'; Waterman 1995). We considered agonistic interactions as those social interactions with the apparent intent of decreasing or ignoring social cohesion among squirrels, including the behaviours run at, chase, slap face, jumped away from, fight, and ritualized fight (Waterman 1995).

As the hours spent observing each social group were not identical, we divided the number of interactions observed per pair of squirrels by the number of hours we observed the pair's social group, resulting in a rate of interactions per hour. We assessed each individuals' most frequent interaction partner, hereafter termed 'primary associate' after Silk et al. (2020), although our designation of primary associate derives from the frequency of all affiliative interactions, rather than from a dyadic sociality index.

Kin biases may be the result of genetic and behavioural isolation by distance, whereby spatially closer squirrels are more closely related due to limited dispersal and, because they are closer, they also interact more frequently (isolation by distance hypothesis; Wright 1943; Meirmans 2012). This explanation does not apply to our study system because we compared interaction frequencies and kinship values within, not among, social groups. Limited dispersal does not apply to individuals who have not dispersed from each other, and the matrilineal kin groups of Cape ground squirrels are the product of female philopatry (Waterman 1995). Observing within social groups thus keeps spatial dispersal constant in our analysis.

Genetic analysis

We used the tissue samples we collected during trapping, preserved in 95% ethanol, for DNA analysis. We extracted DNA using an E.Z.N.A ® Tissue DNA Kit (Omega Bio-tek, Inc., Norcross, GA, U.S.A.) and amplified the DNA with polymerase chain reactions at 20 species-specific microsatellite loci (Abercrombie et al. 2009; Manjerovic & Waterman 2015; Shave & Waterman 2017) in a genetics laboratory at the Assiniboine Park Zoo in Winnipeg, Manitoba. We then sent the DNA to be sequenced at the Centre for Applied Genomics (Hospital for Sick Children, Toronto, Canada). We manually scored each allele using GeneMarker (v2.6.0; SoftGenetics, Inc.; State College, PA, U.S.A.) and checked the data for errors and duplications using GENECAP (v1.4; Wilberg & Dreher 2004). We used COANCESTRY (v1.0.1.8; Wang 2011) with the 'related' (v1.0; Pew et al. 2015) package in R Studio operating on R version 3.5.0 (R Development Core Team 2019) to get pairwise coefficients of relatedness for each pair of

squirrels. We included microsatellite data from 1001 individuals sampled from the study site from 2002 – 2017. As the triadic likelihood estimate *trioml* had the highest correlation with the true data (Wang 2007; Shave & Waterman 2017), we used *trioml* to estimate relatedness using 100,000 bootstrap permutations. Using CERVUS (v.3.0.7; Marshall et al. 1998; Kalinowski et al. 2007), we found no deviations from Hardy-Weinberg equilibrium or linkage disequilibrium with $\alpha = 0.05$ and we assigned parentage to 29 out of the 123 offspring squirrels trapped in 2017. CERVUS uses likelihood estimates to assign parentage with microsatellite data and confidence levels calculated using a simulation of population allele frequencies, the proportion of the population sampled and genotyped, and an estimated mistyping error rate (Manjerovic & Waterman 2015; Marshall et al. 1998). We ran 100,000 iterations in a parent-pair analysis using the proportion of loci successfully typed as calculated from our microsatellite data (0.906) and an assumed 1% genotyping error rate (Manjerovic & Waterman 2015). We used parent-pair analysis rather than maternity analysis because maternity analysis assumes that the fathers are already known (Marshall et al. 1998). We included microsatellite data from 1001 individuals from 2002 – 2017 in the parentage assignments. We included adult females living in the same social group with offspring as potential mothers. We included all adult males on-site as potential fathers (Manjerovic & Waterman 2015).

Statistical analysis

Social network analysis is a behavioural biological tool adapted from human behavioural sciences and computer network theory (Krause et al. 2015). It denotes interactors as *nodes* and their interactions as *edges* (Krause et al. 2009; Farine & Whitehead 2015). Network analysis allows measurement of degree, the total number of edges connected to each node (Farine & Whitehead 2015). Edges can also be weighted, whereby their weight represents an additional variable, such as the frequency or magnitude of interactions (Opsahl et al. 2010). Edges can also be directed or undirected (Farine & Whitehead 2015). Directed edges have a direction of interaction; i.e. one node acts upon another (Farine & Whitehead 2015). Undirected edges have no direction; i.e. the interaction is mutual (Farine & Whitehead 2015). Building networks of nodes and edges allows for tests of properties emergent in structured systems not detectable in their constituent parts (Krause et al. 2015). Network analysis allows for quantifiable, standardized characterization of social structure (Makagon et al. 2012).

We constructed social network models with edges weighted by the frequencies of observed social interactions between squirrels using the *igraph* (v1.2.4.1; Csárdi & Nepusz 2006) and *network* (v1.15; Butts 2008) packages in R Studio operating on R version 4.0.2 (R Development Core Team 2019). We tested for a correlation between observed dyadic affiliative interaction frequencies and agonistic interaction frequencies versus estimates of relatedness as the independent variable by fitting exponential random graph models to our networks with the *ergm* and *ergm.count* (v3.4.0; Hunter et al. 2008; Krivitsky 2016) packages. Exponential random graph models (ERGMs) treat network structure as a dependent variable and statistically test network variables without assuming independence of the residuals, which is an invalid assumption for social networks (Silk & Fisher 2017). ERGMs were originally constructed for unweighted networks but have been expanded to apply to weighted networks (Krivitsky 2012). ERGMs produce fewer false positives than permutation-based null models, especially when modelling dyadic networks of observed interactions, rather than inferred associations using the 'gambit of the group' (Evans et al. 2020; Franks et al. 2010). Results are presented as mean ± SE and an alpha of 0.5 was used to determine significance.

Results

The mean number of squirrels per social group observed was 7.32 ± 0.28 (range = 3 – 12; n = 14 groups). The mean number of females (adults and subadults) per social group was 3.33 ± 0.45 (range = 2 – 7; n = 14), and the mean number of males per social group (natal adults and subadults) was 2.53 ± 0.39 (range = 0 – 5; n = 14). The average relatedness within the social groups observed (0.236 ± 0.016, range = 0.117 – 0.321; n = 291 pairs of squirrels in 14 social groups) was higher than the average relatedness of random assortments of the 14 social groups maintaining their group size and assuming group membership was unrelated to relatedness (0.07 ± 0.001, range = 0.05 – 0.27, n = 100; $t_{(13)} = 9.97$, $P < 0.01$; Figure 1).

We recorded 704 affiliative interactions and 235 agonistic interactions among 92 squirrels in 14 social groups during 518 observation hours. Agonistic interaction frequencies numbered roughly one-third the number of affiliative interactions. Among those interactions, agonistic interactions were overwhelmingly non-contact interactions, like chasing and ritualized fighting. Fewer than 1% of agonistic interactions involved contact (2 / 235); those data excluded interactions with band males.

Among the most frequent interactors within a social group (primary associates), 10 were

offspring-parent pairs, 3 were full siblings, 6 were half-siblings, and 71 were none of the above, (Figure 2). The average relatedness among affiliative primary associate pairs that were not first-degree relatives (parent-offspring pairs, full-siblings) or half-siblings was still higher than the average social group relatedness (Wilcoxon ranked-sum; $W_{(70)} = 2.66 \times 10^7$, $P < 0.0001$). Relatedness did not differ between the most frequent affiliative interaction partners (0.27 ± 0.02) and the second most frequent affiliative interaction partners (0.26 ± 0.02; $t_{(164)} = 0.25$, $P = 0.80$).

We constructed a network of affiliative interactions with 92 nodes and 209 edges (Figure 3). We found a positive relationship between the frequency of affiliative interactions and the degree of relatedness between squirrels within social groups (Monte Carlo maximum likelihood estimate = 12.06; SE = 0.03; $P < 0.0001$). We modeled all combinations of the node variables sex, age, and social group, and the edge variable relatedness as independent variables against the dependent edge variable of interaction ties weighted by frequency. The model with the lowest BIC (Bayesian Inference Criterion) number included all four variables. According to that model, the log-odds of any tie existing were − 0.38 ($P < 0.0001$). The corresponding probability was: e ^ (− 0.38) / (1+e ^ (− 0.38)) = 0.41. The conditional log-odds of two actors having a tie were: − 0.38 × the change in the number of ties + 9.86 × the change in relatedness. For a tie with 0 relatedness, the conditional log-odds are: − 0.38; if relatedness was 1: − 0.38 + 9.86 = 9.48; and the corresponding probabilities are 0.41 and 0.99.

We constructed a network of agonistic interactions with 92 nodes and 146 edges (Figure 4). We found a positive relationship between the frequency of agonistic interactions and the degree of relatedness between squirrels within social groups (Monte Carlo maximum likelihood estimate = 14.82; SE = 0.02; $P < 0.0001$). We modeled all combinations of the node variables sex, age, and social group, and the edge variables relatedness as independent variables against the dependent edge variable of agonistic interaction ties weighted by frequency. The model with the lowest BIC number included only social group and relatedness as independent variables and interaction frequency ties and weight as the dependent variable. Sex and age class were not informative additions to the model of agonistic interactions. According to the model with the lowest BIC, the log-odds of any tie existing was − 1.33 ($P < 0.0001$). The corresponding probability was e ^ (− 1.33) / (1+e ^ (− 1.33)) = 0.209. The conditional log-odds of two actors having a tie were − 1.33 × the change in the number of ties + 14.82 × the change in relatedness. For a tie with 0 relatedness, the conditional log-odds were − 1.33; if relatedness is 1: − 1.33 +

14.82 = 13.49; and the corresponding probabilities are 0.209 and 0.99.

Discussion

Our results found that interactions in Cape ground squirrel social groups were kin-structured. The mean pairwise within-group relatedness was higher than expected from random social group assortments, and Cape ground squirrel social network interactions, excluding those of band males, were biased in favour of closer kin in their social groups both affiliatively and agonistically. Our results also show Cape ground squirrels were more affiliative with same-sex individuals and more affiliative with individuals of a different age class (adult vs subadult) within their social groups. Sex and age class did not seem to influence the network of agonistic interactions. We also found that among the most frequent affiliative interactors within social groups (primary associates), most were not first-order relatives (parent-offspring or siblings) or half-siblings, but their relatedness, on average, exceeded the average within-group relatedness.

Network-level kin biases have been found in highly social animals like elephants (*Loxodonta africana*; Archie et al. 2006), lions (*Panthera leo;* Mosser & Packer 2009), dolphins (*Tursiops aduncus*; Wiszniewski et al. 2010), and many primates (Silk 2002). Nepotism also has important roles in the social structures and fitness outcomes of many mammals, including rodents, and particularly ground-dwelling sciurids (Armitage 1981; Mateo 2003; Hare 2004). Longer-lived mammalian species are predicted to structure their social groups based on kinship more so than shorter-lived species (Davis et al. 2016) and the above-mentioned examples support this hypothesis – male African savanna elephants (Archie et al. 2006), female Indo-Pacific bottlenose dolphins (Wiszniewski et al. 2010), many primate species (Silk 2002), and also coypus (*Myocastor coypus*; Túnez et al. 2009) – as well as short-lived social species that lack kin structure, like woodrats (*Neotoma macrotis*; Matocq & Lacey 2004) and degus (*Octodon degus*; Davis et al. 2016). Cape ground squirrels are a small-bodied species with a potentially long lifespan (living up to 13 years; Zumpt 1970), so the hypothesis that lifespan influences kin structure would predict that Cape ground squirrels structure their social groups based on kinship, and our results add support to that hypothesis (Davis et al. 2016). Like Cape ground squirrels, female Indo-Pacific bottlenose dolphins also preferentially interact with closer kin (Wiszniewski et al. 2010). They also have high female philopatry and live in social groups with both close relatives and non-relatives, and male dolphins do not show kin biases (Wiszniewski et al. 2010). Wiszniewski et al. (2010) hypothesize that kin preferences amongst female dolphins suggest

their nepotistic interactions may provide inclusive fitness benefits. Cape ground squirrels may also bias their interaction networks by kinship for inclusive fitness benefits. To compensate for potential competition among individuals in a group, mammals may direct more of their interactions to kin, effectively forming subgroupings within a larger group (Dunbar 2018). For example, gelada baboons (*Theropithecus gelada*), to decrease the costs of group living, such as competition for resources and decreased female fertility, will direct nepotistic behaviour towards kin and form a subgroup within a larger group (Dunbar 2018). In black-tailed prairie dogs (*Cynomys ludovicianus*), females were nepotistic in their alarm calls for kin (Hoogland 1983). Female black-tailed prairie dogs do not wean a litter until they are 3-4 years old, so younger non-reproductive females in a group will alarm call to protect non-descendent close kin in their coterie, presumably gaining inclusive fitness benefits (Hoogland 1983). Cape ground squirrels are similar in that females often fail to successfully wean young and have only 1-2 offspring at a time (Waterman 1996). Therefore, as adult females within the group will often be without offspring, they have the opportunity to interact with and benefit offspring who are not their own but closely related.

The network of agonistic interactions in Cape ground squirrels showed no bias for age or sex, but agonistic interaction probabilities increased with kinship. Our observation that agonistic interactions increase with closer kin contradicts our prediction that kin selection-driven nepotism would cause agonistic interactions to decrease with closer kin. However, the finding is consistent with the observation that Cape ground squirrel agonistic interactions are relatively mild and infrequent, with few aggressive encounters involving actual contact and no known cases of conspecific mortality, injury, or infanticide (Waterman 1995; this study). Infanticide is otherwise common among ground squirrels (Dobson 1990). In Cape ground squirrels, younger subadult females in the group can be reproductively suppressed, but there is no evidence that the suppression is achieved via aggressive interactions from other individuals (Waterman 2002; Pettitt & Waterman 2011). Like Cape ground squirrels, black-tailed prairie dogs are polygynous cooperative breeders. Black-tailed prairie dogs live in family groups with one adult male, several adult females, and their offspring (Hoogland 1979; King 1955). Black-tailed prairie dogs are nepotistic towards close kin, but their rates of nepotism did not change based on kinship among close relatives; i.e. half-siblings and nephews were interacted with as often as full siblings or offspring, et cetera (Hoogland 1986). Prairie dog groups (coteries) are aggressive and territorial

to neighbouring groups, and their breeding occurs seasonally. During their breeding season, agonistic interactions increase markedly among both males and females within the same coterie (Hoogland 1986). Also, most prairie dog activities occur in a small coterie territory that is defended by adults and yearlings (Hoogland 1986). Female black-tailed prairie dogs are highly social and become aggressive towards each other during mating and pregnancy, especially during their breeding season, as competition for breeding, nest burrows, and other territory is high and elicits aggression (Hoogland 1986). Fights among black-tailed prairie dogs can lead to serious injury and death (Hoogland 1986). Hoogland (1986) argues that because aggression is so costly for black-tailed prairie dogs, it is especially important for them to reduce aggressive behaviour towards kin, and indeed their proportions of affiliative to agonistic interactions show strong nepotism in favour of close kin (Hoogland 1986). Male black howler monkeys (*Alouatta pigra*) similarly had fewer agonistic interactions with closer kin (Van Belle et al. 2014), as did female mountain gorillas (*Gorilla beringei beringei*, Watts 1994). Cape ground squirrels are non-aggressive and non-territorial and most of their interactions occur on the safety of their burrow cluster (Waterman 1995). Female Cape ground squirrels do not compete for nest burrows because there are many vacant burrows in the environment (Waterman 1995; Manno et al. 2007). Cape ground squirrels also breed asynchronously year-round, so females do not compete to raise offspring at the same time as each other, as black-tailed prairie dogs do (Waterman 1996; Hoogland 1986).

The function of agonistic interactions in Cape ground squirrels is unclear. Agonistic interactions may be higher with closer kin simply because interactions overall are higher with closer kin. For yellow-bellied marmots (*Marmota flaviventris*), agonistic interactions positively correlated with affiliative interactions in their social networks, suggesting that the frequencies of both classes of interaction co-vary with an individual's relative position in their social network (Lea et al. 2010). Lea et al. (2010) also found that the relative position of marmots in their agonistic interaction networks positively correlated with their longevity and lifetime reproductive success. The researchers argue that agonistic interactions, if they are mild, can have positive evolutionary consequences, either simply from the correlation with more central social positions or the maintenance of social order (Lea et al. 2010). Yellow-bellied marmots are much less social than Cape ground squirrels and thus may have reduced negative consequences from agonistic social interactions (Lea et al. 2010). However, the low levels of female-female

competition in Cape ground squirrels may translate into the low rates and mild impacts of aggression – where they rarely even contact each other – that we observe in Cape ground squirrels. Such mild agonistic interactions may also not have large fitness costs, and thus greater agonistic interaction frequencies among closer kin may not have negative fitness consequences for closer kin.

Cape ground squirrels also biased their interaction networks by sex, possibly for direct or inclusive fitness benefits. Since females are philopatric while all males eventually leave their natal social group, females may prefer to interact with other females because they will be associating with those same females throughout their lives (Waterman 1995; Mateo 2003; Silk 2009). Female philopatry with male dispersal often correlates with female-kin (matrilineal) interaction biases, including, as reviewed in Silk (2009), among all species of macaques (*Macaca* spp.), savannah baboons (*Papio cynocephalus* spp.) and vervet monkeys (*Clorocebus aethiops*) (Silk 2009). In the above-mentioned primate species, however, the matrilineal biases create matrilineal dominance hierarchies (Silk 2009). Dominance hierarchies are absent in Cape ground squirrels, which is a rare social system (Waterman 1995; Krause et al. 2015). Cape ground squirrels may benefit from matrilineal biases without the influence of dominance hierarchies. Like Cape ground squirrels, African lions live in matrilineal groups (prides) where females live with their offspring (Packer et al. 1991; Mosser & Packer 2009). Lions show sex-biased interactions likely to form coalitions to combat other lion prides to guard territory and protect their offspring (Mosser & Packer 2009). Spotted hyenas (*Crocuta crocuta*) form multi-matrilineal coalitions (clans) often with non-relatives to guard territory against more closely related hyenas in other clans or from lions, an example of direct fitness benefits superseding kinship (Van Horn et al. 2004). These territoriality and infanticide-preventing explanations for sex biased-interactions do not apply to Cape ground squirrel sex biases, because they are not territorial and have not been observed performing infanticide (Waterman 1995; Waterman, pers. comm.). However, female Cape ground squirrels may still form sex-biased coalitions to defend themselves or their offspring from predators or non-territorial threats less severe than death, such as male-biased ectoparasite transmission. Cape ground squirrel females have, on average, lower ectoparasite abundances than males, so females may prefer to interact with other females rather than males to reduce their relative risk of ectoparasite infection (Hillegass et al. 2008). Adult females with emerged juvenile offspring also participate far more often than other sex/age

classes in the predator harassment (mobbing) of snakes (Phillips & Waterman 2014). Among black howler monkeys, males were not more likely to interact with other males (for males, there was no difference in affiliative frequency among males and females); but females were more likely to interact with other females, likely to form coalitions to keep out dispersing females so that resident female sexual attractiveness remains relatively high (Van Belle 2014). This explanation does not apply to Cape ground squirrels, because females do not disperse (Waterman 1995). Female bottlenose dolphins also preferentially interact with other females, perhaps to avoid infanticide, male sexual harassment, or predation, or for cooperative foraging benefits (Wiszniewski et al. 2010). Cape ground squirrel foraging time may increase due to cooperative foraging benefits (Edwards & Waterman 2011), and they most likely group to avoid predation (Waterman 2002).

Cape ground squirrels also biased their interaction networks based on age class within their social groups. Adults were more likely to interact with subadults than with other adults, and vice versa (subadults were more likely to interact with adults than with other subadults). This social structure may be explained by the parental and alloparental care that Cape ground squirrels give to subadult squirrels within their social groups (Manjerovic & Waterman 2015; LaFlèche et al. *Submitted*). About 20% of alloparental care came from adult females in the group that were not the mothers, 20% came from mothers, 60% came from natal males; and since natal males give alloparental care but not parental care, the age structure is unlikely to be solely due to parental interactions (Waterman 1995; LaFlèche et al. *Submitted*). Since the time spent vigilant by individuals increases with increasing distance to the nearest squirrel, associating closely with older squirrels may allow young squirrels to reduce their vigilance time and increase their foraging time (Unck et al. 2009; Waterman 2002; Edwards & Waterman 2011). Natal males do not change their rate of alloparental care relative to how closely related they are to the offspring (LaFlèche et al. *Submitted*). Instead, they likely gain pay-to-stay benefits, where they are allowed to remain in their natal social group and receive benefits from doing so in exchange for providing alloparental care, even though the alloparental care may be costly to the caregivers (LaFlèche et al. *Submitted*). Yellow-bellied marmots also discriminate their interaction frequencies based on age class, but adults interacted more with adults, and subadults interacted more with subadults (Wey & Blumstein 2010). Yellow-bellied marmots do not provide alloparental care (Blumstein & Armitage 1999) but alloparental care is common among Cape ground squirrels. The presence

or absence of alloparental care may help explain why the age structure in Cape ground squirrel social groups is heterophilic while the age structure in yellow-bellied marmots is homophilic (Wey & Blumstein 2010).

Additionally, the kin biases observed in Cape ground squirrel social groups were not due to just parent-offspring interactions, which are expected from direct fitness benefits (Hamilton 1964; Phillips & Tang-Martinez 1998). Most primary associates were squirrel pairs that were not offspring and parents, nor even siblings or half-siblings. The kin bias detected was thus unlikely to be due only to parental care. Adding parent-offspring and sibling relationships to a future social network ERGM would likely confirm that result with greater detail. Prairie voles (*Microtus ochrogaster*) and meadow voles (*M. pennsylvanicus*) are closely related and ecologically similar species, but parent-offspring relations are important to prairie voles and not to meadow voles, likely because prairie voles are mostly monogamous and meadow voles are promiscuous (Oliveras & Novak 1986; Phillips & Tang-Martinez 1998). Among prairie voles, both parents cohabitate and rear their offspring together, and siblings remain with their parents and give alloparental care (Wang & Novak 1992; Silk 2007) – perhaps because monogamy keeps their relatedness reliably high among their family groups (Phillips & Tang-Martinez 1998; Cornwallis et al. 2010). Among meadow voles, fathers do not help rear young, and mothers spend much less time rearing their young than in prairie voles (Getz 1972; McGuire & Novak 1984). Cape ground squirrels are promiscuous, and fathers do not help rear their offspring. However, Cape ground squirrels are facultatively cooperative breeders, and social group members often provide alloparental care (Waterman 1995, 1996; Manjerovic & Waterman 2015). Promiscuity may thus partly explain the relative lack of parent-offspring primary associates. Furthermore, Cape ground squirrels' primary associates were, on average, more related than the study site population's average within-group relatedness: but primary associates were not necessarily the most highly related individuals in their social groups. This finding supports the hypothesis that inclusive fitness benefits, not parental-offspring interactions, leads to Cape ground squirrel kin-biased interactions.

However, our results do not rule out other direct fitness benefits leading to this species' kin-biased interactions. We assumed in our analysis that all social group members were equally free to interact with all other social group members, and thus that any bias in interaction frequencies within social groups was a result of behavioural biases. That assumption likely

applies for Cape ground squirrels but may not apply if there is any correlation between relatedness and any other factor that influences interaction frequencies, such as familiarity (Mateo 2003). If squirrels are more likely to interact with squirrels they have already interacted with more so than others, and if juvenile squirrels are incidentally more likely to interact with closer kin within their social groups despite lacking any behavioural biases, then, as they age, squirrels will still bias their interactions in favour of closer kin despite having no actual nepotism (Mateo 2003). Such familiarity due to spatial sharing or close association is especially applicable to species that are philopatric and share natal nests, especially when juveniles are very young (Holmes & Sherman 1983; Silk 2009). Cape ground squirrels are philopatric and share burrow clusters, but mothers isolate themselves to birth offspring and throughout lactation (Waterman 1996). Mothers only return with their offspring to their natal social group after the offspring are weaned (Waterman 1996). Litter sizes are also small – with only 1–2 offspring birthed at a time – and litter distinctions are not maintained following juvenile emergence (Herzig-Straschil 1978; Waterman 1995, 1996). Thus, there seems to be little opportunity in this species for offspring to form early kin-biased associations with non-parent, non-sibling kin without kin discrimination and nepotism. Cape ground squirrels can recognize kinship from odours on a fine-tuned scale, but they do not discriminate odour-sniffing durations based on kinship with familiar squirrels from within their social group (Waterman & Archibald 2019). Our study shows that Cape ground squirrels behaviourally discriminate based on kinship within their social groups.

In conclusion, Cape ground squirrels biased their interaction networks to interact more often with closer kin within their social groups both affiliatively and agonistically. We had hypothesized that Cape ground squirrels would show behavioural kin biases due to inclusive fitness benefits, predicting that they will interact affiliatively more often and agonistically less often with closer kin within their social groups (Van Belle et al. 2014). We found support for the former but not the latter prediction. Since agonistic interactions are relatively mild in this species, greater agonistic interaction frequencies with closer kin may have insignificant fitness costs. Cape ground squirrels were also more affiliative with group members of their same sex and more affiliative with group members of a different age class (adult vs subadult). Sex biases may be due to direct and indirect fitness benefits associated with predator avoidance and natal philopatry, and age biases may be due to direct fitness benefits associated with pay-to-stay alloparental behaviour. We did not find that sex and age classes influenced their network of

agonistic interactions. We also hypothesized that if kin selection is an important evolutionary mechanism in this species' social interactions, we would find that relatedness was correlated with interaction frequencies beyond just parental care, and we found support for this prediction. Our study shows that Cape ground squirrels are nepotistic with their interactions within their social groups, but the fitness consequences of their interactions remain unknown.

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Tables and figures

Figure 2.1: Histogram of the *expected due to chance* average within-group relatedness of all Cape ground squirrel social groups generated by 100 random assortments maintaining group size and assuming group membership is unrelated to relatedness. The red arrow denotes the *observed* average within-group relatedness of all Cape ground squirrel social groups in 2017.

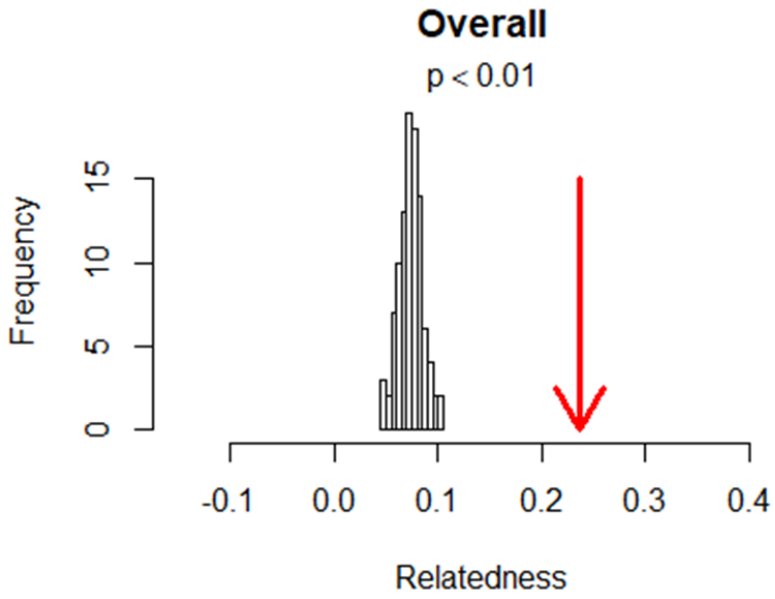


Figure 2.2: Histogram of the number of affiliative primary associate Cape ground squirrel pairs that were also parent-offspring pairs, full-sibling pairs, half-sibling pairs, or none of the above.

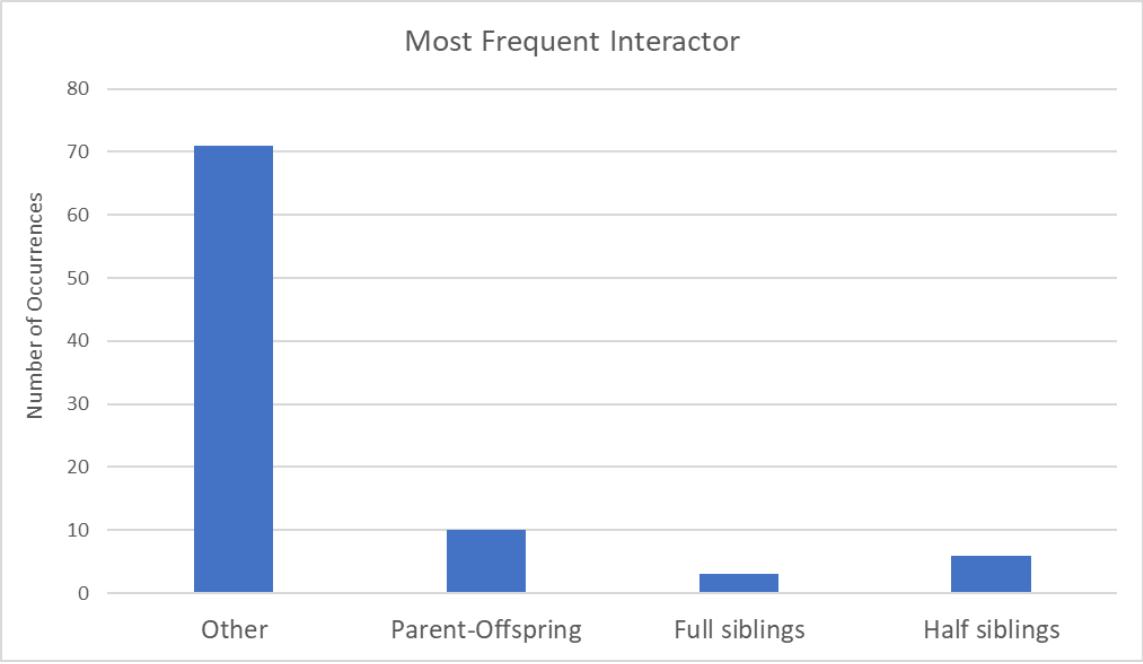


Figure 2.3: Graph of the network of affiliative interactions within fourteen Cape ground squirrel social groups. The circles represent Cape ground squirrels and the lines represent interactions between them.

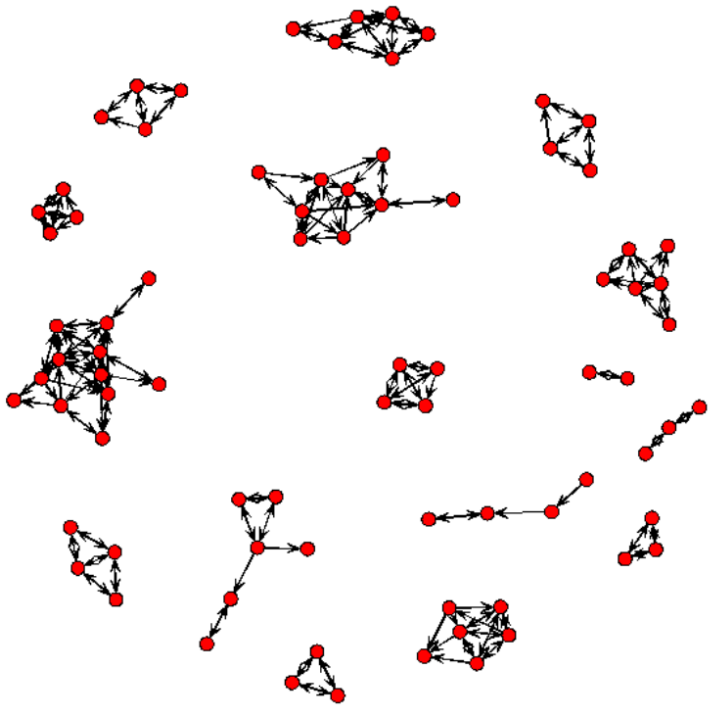
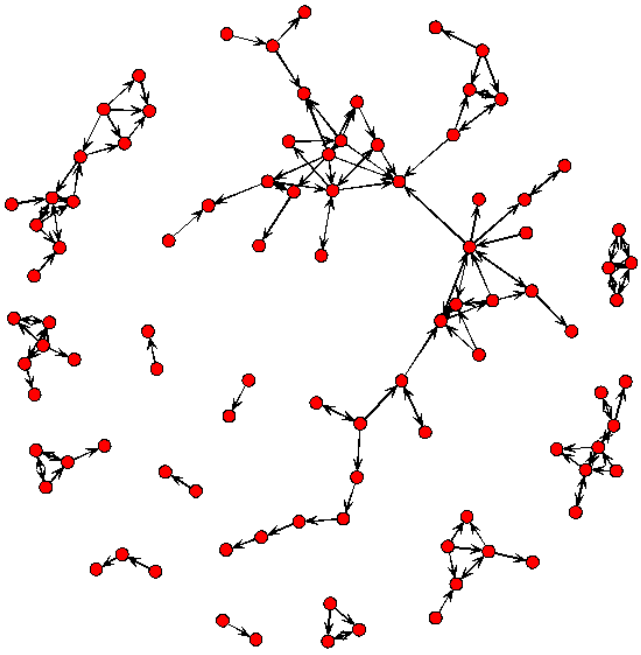


Figure 2.4: Graph of the network of agonistic interactions within fourteen Cape ground squirrel social groups.



Appendix

Figure A.2.1: Diagnostic plots for the best-fit exponential random graph model of the network of affiliative interaction frequencies among Cape ground squirrels.

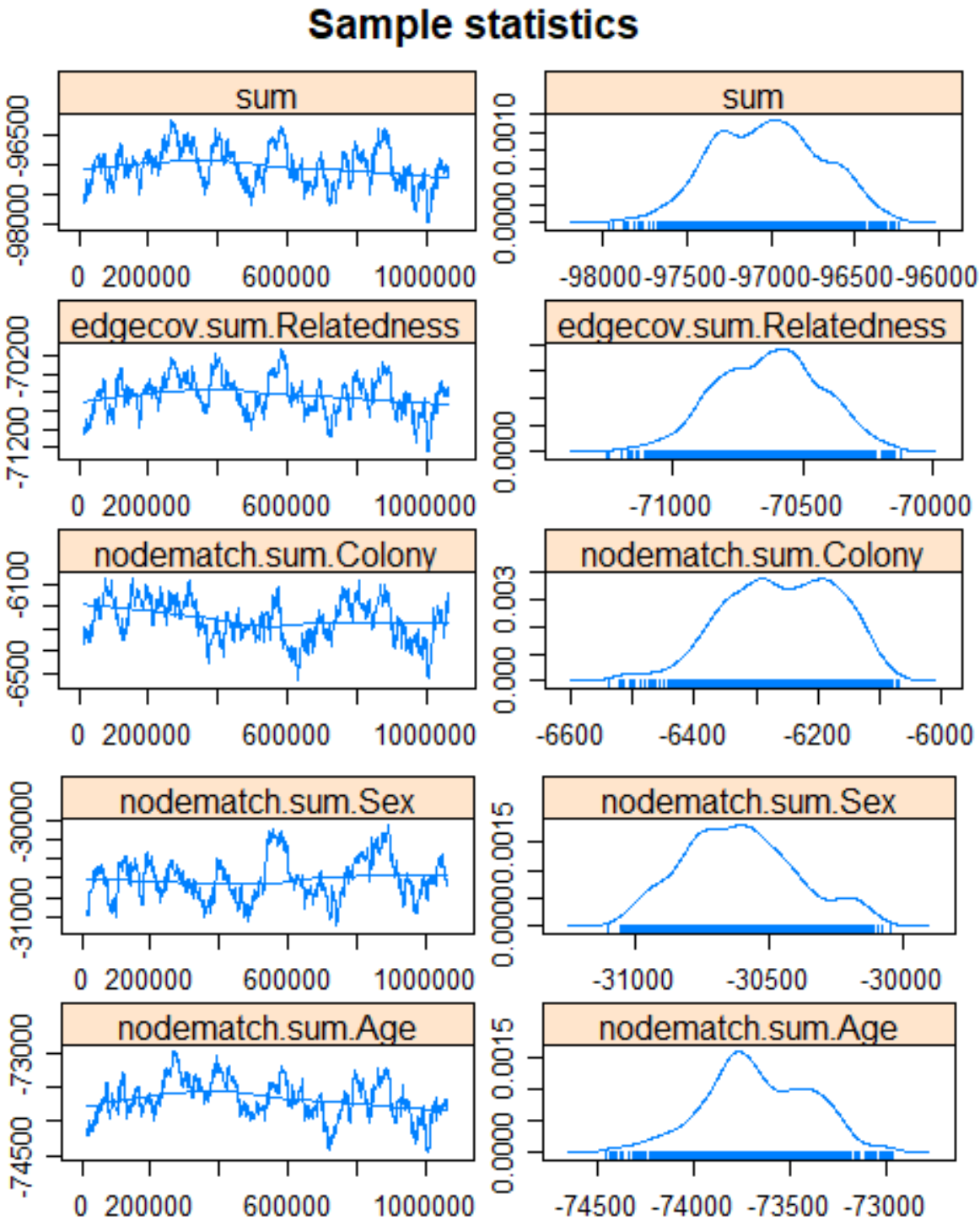
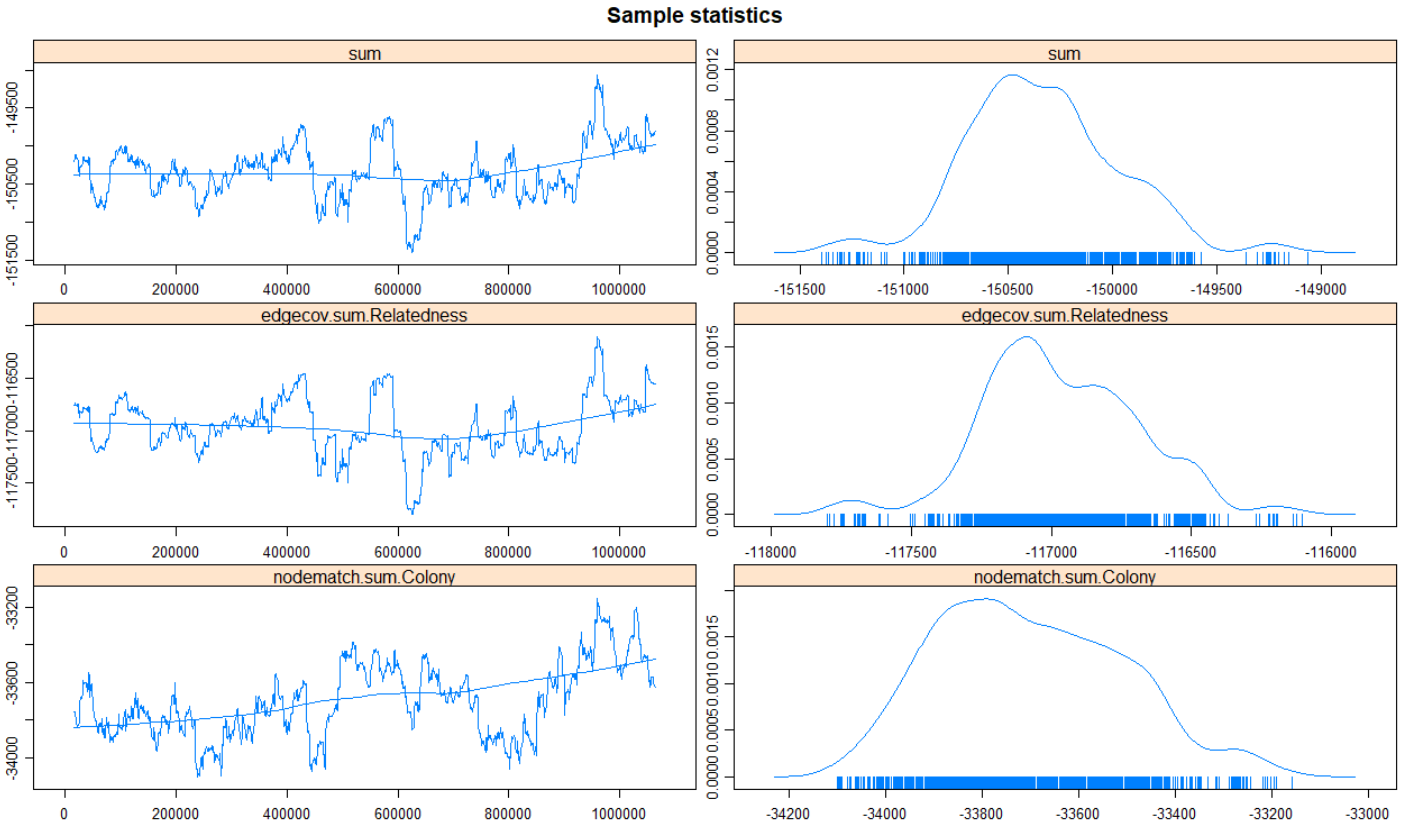


Figure A.2.2: Diagnostic plots for the best-fit exponential random graph model of the network of agonistic interaction frequencies among Cape ground squirrels.



Chapter 3: Variation in centrality, ectoparasites, and reproductive success in Cape ground squirrel (*Xerus inauris*) social networks

Abstract

Exploring whether fitness correlates to social position under low rates of aggressive interactions or in the absence of dominance hierarchies remains a key challenge for comparative evolutionary ecology, as few social species have low rates of aggression. In animal groups, increased social network centrality is often linked to improved fitness, but also to greater parasitic infections. Cape ground squirrels (*Xerus inauris*) are a ground-dwelling species that appear to have a complex social system that lacks the aggression and dominance hierarchies found in many other social species. Previous research showed that experimentally removing parasites from Cape ground squirrels dramatically increased female reproductive success. Our objective was to investigate whether survival and reproductive success are influenced by social centrality and ectoparasite abundance in adult female Cape ground squirrels. We predicted that larger groups will not have higher per capita ectoparasite abundances but will have higher allogrooming frequencies, that more central squirrels will have greater ectoparasite abundance, and that high ectoparasite abundance carries a significant fitness cost to reproductive success but not to survival. We studied Cape ground squirrels on S.A. Lombard Nature Reserve, South Africa in 2017. We followed 14 social groups, collected interaction data through all-occurrence sampling observations, and collected ectoparasite abundance data from trapping. We found that per capita ectoparasites did not increase with larger group sizes but we did not find that allogrooming rates were related to group size. Adult females with greater eigenvector centrality scores had higher ectoparasite abundances, but there was no significant fitness cost to having greater eigenvector centrality or ectoparasite abundance. Our study shows understanding the costs and benefits of group living in species with low rates of aggression can have novel implications for the relationships between fitness and sociality.

Introduction

Sociality has evolved in all major mammalian lineages and across broad ecological and evolutionary contexts, suggesting strong selection for this behaviour (Smith et al. 2017). Fitness costs and benefits specific to individuals explain why social behaviour evolves (Alexander 1974; Silk 2007; Clutton-Brock 2009). Living alongside conspecifics can increase competition for resources and reproductive opportunities, attract predator attention, and expedite disease and parasite transmission, among other costs (Kutsukake & Clutton-Brock 2006; Treisman 1975; Silk et al. 2018; Wey & Blumstein 2012). The fitness benefits of sociality may include enhanced predator avoidance, improved mate access, communal habitat alteration, thermal conservation, reciprocal parasite removal, cooperative breeding, and communal territorial defense, among other benefits (van der Marel 2019; Altizer et al. 2003; Johnson et al. 2004; Garroway et al. 2013; Mosser & Packer 2009; Pulliam 1973; Treisman 1975; Bertram 1978; Hamilton 1971). Thus, conspecifics must navigate between the positive and negative effects of groupmates to maximize their fitness.

Intragroup social interactions are considered one of the fundamental components determining social complexity (Kappeler 2019). Traditional analyses of paired (dyadic) social interactions are informative but insufficient to characterize the structure of complex social groups (Makagon et al. 2012; Krause et al. 2015). Social network analysis allows consideration of indirect social connections (Newman 2003; Krause et al. 2015). Centrality measurements are one class of network metric that quantify the relative importance of an individual within a network of social interactions, allowing social position to be quantitatively compared to other individual variables, such as fitness measures (Newman 2003; Krause et al. 2015). Eigenvector centrality measures the interaction degree (the number of interactors) of an individual weighted by the degree of those interactors in turn (Newman 2003; Bonacich 2007; Makagon et al. 2012; Cheney et al. 2016). Social network position may have evolutionarily important fitness consequences when a relationship exists between centrality and reproductive success (Brent 2015). More favourable network positions correlate positively to individual longevity and fitness estimates in a variety of animal taxa (Formica et al. 2012; Farine & Sheldon 2015; Silk et al. 2010; Cheney et al. 2016; Schülke et al. 2010; Cameron et al. 2009; Natoli et al. 2007; von Holst et al. 2002; Yee et al. 2008; Wey & Blumstein 2012; Stanton & Mann 2012). Animals that are more central in social networks may gain fitness benefits and fitness costs that are similar to the

benefits and costs of social living generally, but especially those benefits and costs that arise from indirect social connections (Makagon et al. 2012; Brent 2015). For example, if information about approaching predators passes between socially connected animals, it would be more beneficial for individuals to connect with well-connected animals than connecting to an animal without those other connections (Evans et al. 2020).

However, high rates of direct and indirect interactions can also be costly (Krause et al. 2015; Evans et al. 2020). One cost of high interactions is the potential to transmit infections and parasites (Godfrey 2013; Lucatelli et al. 2020). In animal groups, increased social network centrality is often linked to improved fitness, but also to greater ectoparasite abundance (Brent 2015; Godfrey 2013). Parasites vary in the significance of their fitness costs, but the costs can become severe (Patterson et al. 2013; Akinyi et al. 2019). If costly parasites pass between animals, high rates of interaction between more connected (central) individuals should be more costly than interactions with less central individuals (Evans et al. 2020; Silk et al. 2018). As social group size increases, the total parasites and parasites per individual may increase (Hoogland 1979; Brown & Brown 2004; Rifkin et al. 2012). Larger groups can increase parasite abundance if larger groups experience more interactions among conspecifics, or by increasing host density and thus availability for parasites, or if larger groups of animals are more noticeable to mobile parasites or their vectors (Dunbar 1991; Rifkin et al. 2012; Nunn & Heymann 2005). Animals may mitigate the increasing abundance of ectoparasites in larger social groups by removing ectoparasites from each other, termed allogrooming (the grooming of other individuals), a strongly affiliative behaviour (Kutsukake & Clutton-Brock 2006).

If ectoparasites are spread via social contact, it follows that greater social contact may increase an individual's ectoparasite abundance (Altizer et al. 2003). Furthermore, greater social contact with individuals who themselves have greater social contact is likely to increase an individual's ectoparasite abundance even more so (Brent 2015). The summed weight of such direct and indirect social connections can be measured with the social network metric termed eigenvector centrality (Bonacich 2007; Cheney et al. 2016; Viblanc et al. 2016). More central individuals have higher parasite abundances in a variety of taxa, including reptiles (pygmy bluetongue lizards, *Tiliqua adelaidensis*, Fenner et al. 2011; sleepy lizards, *Tiliqua rugosa*, Leu et al. 2010; and tuatara, *Sphenodon punctatus*, Godfrey et al. 2010) and mammals (macaque macaques, *Macaca fuscata yakui*, MacIntosh et al. 2012, but see Duboscq et al. 2016; brown

spider monkeys, *Ateles hybridus*, Rimbach et al. 2015; giraffes, *Giraffa camelopardalis*, VanderWaal et al. 2014; and brushtail possums, *Trichosurus vulpecula*, Corner et al. 2003). Ectoparasites are also often more likely to infect individuals who physically contact others, individuals who allogroom, and individuals who associate with infected individuals (MacIntosh et al. 2012; Rimbach et al. 2015; White et al. 2017).

Researchers often divide social interactions into affiliative and agonistic interactions (Lea et al. 2010). Affiliative and agonistic interactions often have different consequences (Wey & Blumstein 2010; Gould 1997; Overduin-de Vries et al. 2020) and fitness advantages from network positions are often correlated to favorable positions in dominance hierarchies established by overt agonistic interactions (Krause et al. 2015). Exploring whether fitness correlates to social position under low rates of aggressive interactions or in the absence of dominance hierarchies remains a key challenge for comparative evolutionary ecology, as few social species have low rates of aggression (Krause et al. 2015, Overduin-de Vries et al. 2020).

Cape ground squirrels (*Xerus inauris*) are a social species that perform high rates of affiliative interactions, particularly allogrooming (Hillegass et al. 2010; Pettitt & Waterman 2011; Waterman 1995). They rarely exhibit aggressive behaviours towards conspecifics and show no evidence of dominance hierarchies (Waterman 1996). Multiple females within each social group breed, and they are facultatively cooperative breeders (Waterman 1995, 1996; Manjerovic & Waterman 2015). Allogrooming does not appear to be related to social status or social dominance behaviour as seen in many primates or habituated meerkats (*Suricata suricatta*), but instead appears to function solely to remove ectoparasites (Spruijt et al. 1992; Kutsukake & Clutton-Brock 2006; Hillegass et al. 2010). Ectoparasite abundance did not increase with group size among Cape ground squirrel social groups (Hillegass et al. 2008). Experimentally removing parasites from Cape ground squirrels substantially increased female reproductive success without changing their litter size or body mass (Hillegass et al. 2010). The per capita number of weaned offspring was four times higher in treated versus untreated females (Hillegass et al. 2010). Although all adult females in Cape ground squirrel social groups attempt reproduction, fewer than half are successful at raising offspring to emergence each year (Waterman 1996). This reproductive skew among females suggests breeding competition occurs, despite a lack of aggression, territoriality, and overt dominance (Pettitt & Waterman 2011; Waterman 2002). Immature females are reproductively suppressed via delayed maturity in the

presence of more than three adult female group members, but there is no evidence for reproductive suppression among adult females (Pettitt & Waterman 2011; Waterman 2002). The reason for the stark differences in reproductive success among adult females remains unknown.

The objective of our study was to investigate whether survival and reproductive success are influenced by ectoparasite abundance and centrality in adult female Cape ground squirrels. We hypothesized that Cape ground squirrels mitigate rising numbers of ectoparasites in larger groups by allogrooming more often, predicting that larger groups will not have higher per capita ectoparasite abundances but will have higher allogrooming frequencies. We also hypothesized that greater direct and indirect social connection among female Cape ground squirrels increases their ectoparasite infection rates, predicting that squirrels with greater affiliative eigenvector centrality will have greater ectoparasite abundance. We further hypothesized that high ectoparasite abundance carries a significant fitness cost to reproductive success but not to survival, since the removal of parasites decreased Cape ground squirrel adult females' number of weaned offspring but did not affect their (the mothers') body mass (Hillegass et al. 2010). We predicted that individuals with greater ectoparasite abundance and greater centrality will have fewer surviving offspring but have no change in their survival.

Methods

Study site

We collected data from April to August 2017 in central South Africa at S.A. Lombard Nature Reserve (3660 ha, 18 km northwest of Bloemhof, North West Province, 27º35'S, 25º23'E), an arid grassy floodplain ecosystem dominated by the tall perennial grasses (van Zyl 1965). Cape ground squirrels have been studied at this site since 2002 (Scantlebury et al. 2007). The site has an average annual rainfall of 502 mm with a range of 241–965 mm from the years 1952–2004 (Pettitt & Waterman 2011). The annual rainfall on the site in 2017 was 552 mm.

Biology of the Cape ground squirrel

Cape ground squirrels are diurnal, semi-fossorial, and primarily herbivorous (Herzig-Straschil 1978). Social groups emerge from their burrow clusters in the morning to forage on the stems, leaves, and seeds of grasses and return to their burrows before dusk (Herzig-Straschil 1978; Skurski & Waterman 2005). They dig groups of burrows connected underground in distinct clusters isolated from other nearby burrow clusters, and only a single social group sleeps

together in a burrow cluster (Waterman 1995). We define a social group as a group of adult females, subadult (immature) females, subadult (immature) males, and natal males – males that delay dispersal – that are observed to rise together from a single burrow cluster in the morning and return together to a single burrow cluster in the evening (Waterman 1995; Herzig-Straschil 1978). Social group members often provide alloparental care, the care of non-descendant offspring (Waterman 1995, 1996; Manjerovic & Waterman 2015; LaFlèche et al. *Submitted*). Males either disperse at maturity or delay dispersal for up to four years (natal males; Manjerovic & Waterman 2015). After males disperse from their natal social groups, they roam widely in all-male bands (band males) in search of females in estrus (Waterman 1995, 1996, 2002). Females enter estrus year-round and spontaneously (Bouchie et al. 2006) and mate with multiple males (Waterman 1998). Social groups have semi-stable residences in a single burrow cluster, while band males form ephemeral fission-fusion bands that are transient throughout the floodplain ecosystem, sleeping in vacant burrow clusters (Waterman 1995; Scantlebury et al. 2008).

Ectoparasites of Cape ground squirrels include lice (e.g. *Neohaematopinus faurei*), fleas (e.g. *Synosternus caffer*) and ticks (e.g. *Rhipicephalus theileri*) (Straschil 1975; Lynch 1983; Waterman 2002; Scantlebury et al. 2007). Ticks, however, are rare on Cape ground squirrels at this study site (Beaumont et al. 2019). Male Cape ground squirrels carry on average three times more ectoparasites than females (Hillegass et al. 2008). Subadult males carry more ectoparasites than subadult females even though they receive the same amount of allogrooming, suggesting hormonal sex differences as the cause of the sex bias in ectoparasite abundance (Hillegass et al. 2008; but see O'Brien et al. 2018 and Bilbo & Nelson 2001).

Trapping and measurements

We trapped squirrels with Tomahawk live traps (Tomahawk Live Trap Inc., WI; 15 X 15 X 50 cm) baited with peanut butter mixed with crushed bird seed. We then transferred the squirrels to cone-shaped handling bags (Koprowski 2002) that allowed us to record body measurements, including mass, foot length, spine length, and reproductive condition. We considered females to be adults when they started breeding, indicated by nipples that are visibly swollen (nipples swell permanently at first estrus; Pettitt & Waterman 2011) or extended from lactation, or if their vulva is swollen, which indicates estrus (Waterman 1996; Pettitt et al. 2008). Females can reach sexual maturity at 8 months when living with only one adult female in their social group or as late as 14 months when living with multiple adult females (Pettitt & Waterman

2011). We determined males were adults if their testes had descended into their external scrotum (adult males are scrotal year-round), which occurs at 8 months (Waterman 1996). Immature squirrels do not have swollen nipples or descended testicles (Waterman 1996), and we classified squirrels without swollen nipples or descended testicles as subadults (Waterman 1995; Pettitt et al. 2008). We injected each squirrel with a passive integrated transponder (PIT tag; AVID Inc., Norco, CA, U.S.A.) for permanent unique identification, created a small freeze mark (Lectro-Freeze Communica Ltd., Pretoria, South Africa; Rood & Nellis 1980) for permanent identification at a distance, and painted a large unique dorsal dye mark (Rodol D, Lowenstein and Sons Inc., New York, NY, U.S.A.) for temporary individual identification at a distance (Waterman 2002). We also collected a small amount of skin (1-3mm) from the tail tip of each squirrel as tissue samples and preserved them in 95% ethanol for later DNA extraction and analysis (see below).

During trapping, we counted and collected ectoparasites (fleas, ticks, and lice) we observed on the squirrels. We examined the groin and inner thighs and combed through the fur on their back three times per body plane (left, right, and middle) with a flea comb (Scantlebury et al. 2007; Hillegass et al. 2008; Beaumont et al. 2019). We immediately preserved the ectoparasites in 95% ethanol and recorded their number (Scantlebury et al. 2007; Hillegass et al. 2008). We used the total number of ectoparasites observed on each squirrel during their first capture in 2017 as an estimate of their ectoparasite abundance (Scantlebury et al. 2007; Hillegass et al. 2008). We released all squirrels at their capture site. Our methods followed the 2016 Guidelines of the American Society of Mammalogists (Sikes 2016). The University of Manitoba's Animal Care and Use Committee (F14-032) approved all research protocols.

Behavioural observations

We recorded all squirrel social interactions during 518 observation hours in 2017 (all-occurrences sampling; Altmann 1974). We defined social interactions by an ethogram described in Waterman (1995). We divided social behaviours into affiliative interactions ('socially cohesive'), and agonistic interactions ('socially competitive'; Wey & Blumstein 2010; Lea et al. 2010). We considered affiliative interactions as those social interactions with the apparent intent of increasing or maintaining social cohesion among squirrels, including allogrooming, touching snouts or 'greeting', approaching, playing, following, and rolling over to solicit allogrooming ('presenting'; Waterman 1995). We considered agonistic interactions as those social interactions

with the apparent intent of decreasing or ignoring social cohesion among squirrels, including the behaviours run at, chase, slap face, jumped away from, fight, and jumping in the air past each other or 'ritualized fight' (Waterman 1995). We also recorded the direction of the interactions; i.e. who interacted with whom. We used 10 x 50mm binoculars and 15 - 45 x 60mm spotting scopes (Spacemaster, Bushnell Co. KS, U.S.A.) and concealed ourselves atop of towers and vehicle hides 50 to 100m from the squirrel burrow cluster. We focused our observation sessions on one or two focal social groups chosen with a random number generator per session but recorded all squirrels visible to us. We did not include days of estrus in our analysis, because males and females behave differently on those days, and we excluded band males from our analyses, as they do not live with the females (Waterman 1995, 1997, 1998).

Genetic analysis

We estimated reproductive success for adult females as their number of offspring in 2017 detected by DNA analysis of the squirrel population trapped in 2017 (Manjerovic & Waterman 2015). From the tissue samples collected from each squirrel in the field, we extracted DNA using an E.Z.N.A ® Tissue DNA Kit (Omega Bio-tek, Inc., Norcross, GA, U.S.A.), amplified the DNA with polymerase chain reaction (PCR), and fluoresced 20 species-specific microsatellite loci (Abercrombie et al. 2009; Manjerovic & Waterman 2015; Shave & Waterman 2017) in the genetics laboratory at the Assiniboine Park Zoo in Winnipeg, Manitoba. We sent the DNA to be sequenced at the Centre for Applied Genomics (Hospital for Sick Children, Toronto, Canada). We manually scored each allele using GeneMarker (v2.6.0; SoftGenetics, Inc.; State College, PA, U.S.A.) and checked the microsatellite data for errors, duplications, and the number of typed loci using GENECAP (v1.4; Wilberg & Dreher 2004). We genotyped 241 individuals, but we removed 4 samples because they were missing at least 10 of the 20 loci. We set the maximum threshold at 10 loci missing because at that threshold we eliminated all mismatched genotypes. Using CERVUS (v.3.0.7; Marshall et al. 1998; Kalinowski et al. 2007), we found no deviations from Hardy-Weinberg equilibrium or linkage disequilibrium with $\alpha = 0.05$, and we assigned parentage to 29 offspring out of the 123 subadult squirrels trapped in 2017. CERVUS uses likelihood estimates to assign parentage with microsatellite data and confidence levels calculated using a simulation of population allele frequencies, the proportion of the population sampled and genotyped, and an estimated mistyping error rate (Manjerovic & Waterman 2015). We ran 100,000 iterations in a parent-pair analysis using the proportion of loci successfully typed as

calculated from our microsatellite data (0.906) and an assumed 1% genotyping error rate (Manjerovic & Waterman 2015). We used parent-pair analysis rather than maternity analysis because maternity analysis assumes that the fathers are already known (Marshall et al. 1998). We included microsatellite data from 1001 individuals from 2002 – 2017 in the parentage assignments. We included adult females living in the same social group with offspring as potential mothers. We included individuals that were subadults in 2017 or 2016 as potential offspring. We included all adult males on-site in 2017 and 2016 as potential fathers (Manjerovic & Waterman 2015).

Statistical analysis

Social network analysis allows for tests of centrality, a measure of interconnectedness within social networks (Newman 2003; Makagon et al. 2012; Krause et al. 2015). Network analyses denote interactors as nodes and their interactions as edges (Newman 2003; Farine & Whitehead 2015). Edges can be weighted, whereby their weight represents an additional variable, such as the frequency or magnitude of interactions (Newman 2003; Farine & Whitehead 2015). Edges can also be directed or undirected (Newman 2003; Farine & Whitehead 2015). Directed edges have a direction of interaction; i.e. one node acts upon another (Newman 2003; Farine & Whitehead 2015). Undirected edges have no direction; i.e. the interaction is mutual (Newman 2003; Farine & Whitehead 2015). Building networks of nodes and edges allows for tests of properties emergent in structured systems not detectable in their constituent parts (Makagon et al. 2012; Krause et al. 2015). Social network analyses of Cape ground squirrels have not been published before, and our analysis characterizes their social structure in quantifiable, standardized terms (Makagon et al. 2012).

We divided the interactions between squirrels into affiliative and agonistic interactions and summed them for each individual (Harris & White 1992; Videan et al. 2005; Lea et al. 2010). As the hours spent observing each social group were not identical, we divided the number of interactions observed per squirrel by the number of hours we observed the squirrel's social group, resulting in a rate of interactions per hour (Waterman 2002). We conducted all our statistical analyses in R Studio operating on R version 4.0.2 (R Development Core Team 2019). We constructed social network models with edges weighted by the frequencies of observed social interactions between squirrels using the *igraph* (v1.2.4.1; Csárdi & Nepusz 2006) and *network* (v1.15; Butts 2008) packages. We fit exponential random graph models to our networks

with the *ergm* and *ergm.count* (v3.4.0; Hunter et al. 2008; Krivitsky 2016) packages. Exponential random graph models treat network structure as a dependent variable and statistically test network variables without assuming independence of the residuals, which is an invalid assumption for social networks (Silk & Fisher 2017). Exponential random graph models were originally constructed for unweighted networks but have been expanded to apply to weighted networks (Krivitsky 2012). We calculated eigenvector centrality scores based separately on affiliative and agonistic interactions for each adult female.

We estimated the age of adult females from our long-term dataset. We used our assessments of sexual maturity and body mass at the time of their first capture to determine each squirrel's time of birth to within one year (Pettitt & Waterman 2011). We assessed survival as the number of years the adult females were observed again in 2018 and 2019. We estimated reproductive success as the number of offspring each adult female had in 2017, as determined by genetic analysis (Manjerovic & Waterman 2015). We used a Kruskal-Wallis $\chi 2$ test to analyse differences in affiliative and agonistic interaction frequencies and eigenvector centralities for different age-sex classes, as our data violated the equality of variance and normality assumptions. If the Kruskal-Wallis test showed significant differences, we used a post-hoc Wilcoxon rank-sum test to test which groups differed from each other. We used linear regressions to test for relationships between fitness estimates – age, survival, and the number of offspring – versus individual attributes: affiliative and agonistic interaction frequencies and eigenvector centralities. Table 3.1 outlines the dependent and independent variables. We used the *lme4* (v1.1-23; Bates et al. 2015) and *lmerTest* (v3.1-3; Kuznetsova et al. 2017) packages to fit generalized linear mixed models (GLMMs) with negative binomial distributions and social group as a random effect, since ectoparasite data were not normally distributed, and since we took multiple measures from each social group (Silk et al. 2020). Negative binomial distributions best fit the ectoparasite data and minimized skew, excess kurtosis, and heteroscedasticity of the residuals. We reported Wald-Z statistics when comparing ectoparasite abundance to group size, interaction and allogrooming frequencies, and network scores. We assessed and validated our GLMMs using the *DHARMa* package (Hartig 2020). Results are presented as mean ± SE, and we used an alpha value of 0.05 to determine significance.

Results

The mean social group size was 7.32 ± 0.28 (range = 3 – 12; *n* = 14 groups) individuals. The mean number of females (adults and subadults) per social group was 3.33 ± 0.45 (range = 2 – 7; *n* = 14), and the mean number of males per social group (natal adults and subadults) was 2.53 ± 0.39 (range = 0 – 5; *n* = 14). The mean total ectoparasite abundance on social group squirrels was 20.21 ± 5.40 (range = 0 – 71; *n* = 14 social groups) ectoparasites (fleas, ticks, and lice). The mean per capita ectoparasite abundance on social group squirrels was 3.16 ± 0.39 (range = 0 – 15; *n* = 90 squirrels) ectoparasites, and the mean ectoparasite abundance on adult females was 2.60 ± 0.58 (range = 0 – 11; *n* = 25 squirrels). The mean rate of allogrooming received by adult females was 0.096 ± 0.022 (range = 0 – 0.455; *n* = 25 squirrels) interactions per observation hour. The mean rate of allogrooming given by adult females was 0.125 ± 0.0255 (range = 0 – 0.453; *n* = 25 squirrels) interactions per observation hour. The mean affiliative interaction frequency of adult females was 0.334 ± 0.057 (range = 0 – 0.931; *n* = 25 squirrels) interactions per observation hour. The mean agonistic interaction frequency of adult females was 0.178 ± 0.034 (range = 0 – 0.732; *n* = 25 squirrels) interactions per observation hour. The mean affiliative eigenvector centrality score of adult females was 1.52e-17 ± 3.67e-18 (range = 0 – 7.23e-17; *n* = 25 squirrels). The mean agonistic eigenvector centrality score of adult females was 0.009 ± 0.009 (range = 0 – 0.233; *n* = 25 squirrels) interactions per observation hour. The mean age of adult females was 2.48 ± 0.28 (range = 1 – 7, *n* = 25 adult females) years. The mean survival of adult females after 2017 was 1.4 ± 0.15 (range = 0 – 2, *n* = 25 adult females) years. The per capita number of offspring per adult female in 2017 was 1.04 ± 0.20 (range = 0 – 3, *n* = 25 adult females).

We found no relationship between per capita ectoparasite abundance and group size (negative binomial GLMM, estimate = 0.10, SE = 0.095, Z = 1.06, df = 86, P = 0.29), but total social group ectoparasite abundance did increase with group size (negative binomial GLMM, estimate = 0.22, SE = 0.097, Z = 2.23, df = 10, P = 0.026). We found no relationship between allogrooming frequency and group size (negative binomial GLMM, estimate = -0.14, SE = 0.13, Z = -1.07, df = 86, P = 0.28). We found no relationship between the rate of allogrooming received and the ectoparasite abundance on the squirrel receiving the allogrooming among adult females (negative binomial GLMM, estimate = 1.34, SE = 2.31, Z = 0.58, df = 21, P = 0.56). We also found no relationship between the rate of allogrooming given and the ectoparasite

abundance on the squirrel giving the allogrooming among adult females (negative binomial GLMM, estimate = 0.28, SE = 2.22, $Z = 0.13$, df = 21, $P = 0.90$). Ectoparasite abundance increased with affiliative eigenvector centrality among adult females (negative binomial GLMM, estimate = 0.31, SE = 0.088, $Z = 3.46$, df = 21, $P < 0.001$; Figure 3.1). Ectoparasite abundance did not decrease with increasing agonistic eigenvector centrality among adult females (negative binomial GLMM, estimate = -0.178, SE = 0.105, $Z = -1.69$, df = 21, $P = 0.091$).

The mean affiliative interaction frequency in social groups observed did not differ between females (0.36 ± 0.04, $n = 52$) and males (0.46 ± 0.07, $n = 38$; $t = -1.29$, df = 88, $P = 0.20$). The mean affiliative eigenvector centrality in social groups observed also did not differ between females (0.007 ± 0.006, $n = 52$) and males (0.04 ± 0.02, $n = 38$; $t = -1.50$, df = 88, $P = 0.14$). In addition, affiliative interaction frequencies did not differ amongst sex and age classes (Kruskal-Wallis $\chi 2 = 3.00$, df = 3, $P = 0.39$). The mean affiliative eigenvector centrality among social groups (females, subadult males, and natal males) was 0.02 ± 0.01 (range = 0 – 0.68; n = 90 squirrels in 14 social groups). Affiliative eigenvector centrality scores did differ amongst sex and age classes (Kruskal-Wallis $\chi 2 = 9.95$, df = 3, $P = 0.019$). The mean affiliative eigenvector centrality of the subadult males (6.55E-02 ± 3.93E-02 , range = 0 – 6.80E-01, $n = 23$) was greater than that of the adult females (1.52E-17 ± 3.67E-18, range = 0 – 7.23E-17, $n = 25$); post-hoc comparison: pairwise Wilcoxon rank-sum, $P = 0.031$). We found no difference between the mean affiliative eigenvector centrality of any other sex-age class: adult females and subadult females (1.33E-02 ± 1.33E-02, range = 0 – 0.35, $n = 26$ subadult females; $P = 0.146$); adult females and adult males (1.21E-03 ± 1.21E-03, range = 0 – 1.82E-02, $n = 15$ adult (natal) males; $P = 0.234$); subadult females and adult males ($P = 0.507$); subadult males and adult males ($P = 0.146$); or subadult females and subadult males ($P = 0.234$).

We also found affiliative interaction frequencies were not affected by age ($F_{(1, 24)} = 3.89$, $P = 0.06$) and did not affect survival ($F_{(1, 24)} = 0.33$, $P = 0.57$) or number of offspring ($F_{(1, 24)} = 0.05$, $P = 0.82$) in adult females (Table 3.1).We found affiliative eigenvector centrality scores were not affected by age ($F_{(1, 23)} = 0.53$, $P = 0.48$), and did not affect survival ($F_{(1, 23)} = 1.04$, $P = 0.32$) or number of offspring ($F_{(1, 23)} = 0.28$, $P = 0.60$) in adult females (Table 3.1; Figure 3.2). We also found agonistic interaction frequencies and agonistic eigenvector centrality scores were not affected by age and did not affect survival or number of offspring in adult females (see Table 3.1).

Discussion

Our results supported the prediction that social group size is not related to average ectoparasite abundance but did not support the prediction that allogrooming rates are related to group size. Eigenvector centrality was correlated with ectoparasites in adult female Cape ground squirrels. Our results did not support the prediction that ectoparasite abundance or eigenvector centrality correlated with estimates of reproductive success but supported the prediction that ectoparasite abundance and eigenvector centrality would not correlate with survival.

We found no relationship between per capita ectoparasite abundance and group size, confirming an earlier study at the same site that also found no relationship between group size and ectoparasite abundance in Cape ground squirrels (Hillegass et al. 2008). The sum of ectoparasites in social groups did rise with increasing group size. Increasing parasite abundance is a typical cost of group living and increasingly so as social group sizes increase (Rifkin et al. 2012). As social group size increases, the total parasites and parasites per individual are predicted to increase (Rifkin et al. 2012). Larger groups can increase parasite abundance via more interactions, greater host density, and greater detectability to mobile parasites or their vectors (Dunbar 1991; Rifkin et al. 2012; Nunn & Heymann 2005). A larger number of host individuals and shared host den sites also reduces the chances that a population of parasites will be eliminated stochastically (Johnson et al. 2004). Animals may mitigate the increasing abundance of ectoparasites in larger social groups by grooming ectoparasites from themselves (autogrooming) and from each other (allogrooming) (Mooring & Hart 1992; Johnson et al. 2004; Hawlena et al. 2006). Parasite removal is the primary cause of grooming behaviour in gerbils (*Meriones crassus*), brown rats (*Rattus norvegicus domestica*), and European badgers (*Meles meles*) (Hawlena et al. 2007; Hart 1992; Johnson et al. 2004). Experimental removal of ectoparasites almost eliminated both autogrooming and allogrooming behaviour in Cape ground squirrels, suggesting their grooming behaviour is also stimulus-driven by the presence of ectoparasites (Hillegass et al. 2010). Stimulus-driven grooming contrasts with dominance behaviours described in several primates (Schino 2001), with social stress relief in habituated suricates (Madden & Clutton-Brock 2009), and with the programmed grooming behaviour model described in several ungulates, where autogrooming behaviour is timed to remove ectoparasites preventatively (Mooring et al 2006a; Mooring et al. 2006b). Both autogrooming and allogrooming appeared to specifically target ectoparasite stimuli in Cape ground squirrels and

may explain why larger groups did not have more ectoparasites per capita (Hillegass et al. 2008). We did not find that allogrooming increased with ectoparasite abundance, but we did not test whether autogrooming rates changed with ectoparasite abundance or group size.

Seasonality may also impact parasite abundance and grooming tendencies. Unlike Cape ground squirrels, for female Japanese macaques (*Macaca fuscata fuscata*), degree centrality was negatively associated with lice abundance, evidence supporting that macaque allogrooming behaviour is effective at removing lice (Duboscq et al. 2016). However, female Japanese macaque degree centrality was only negatively associated with lice abundance in reproductively active females during their mating and breeding seasons (Duboscq et al. 2016). Cape ground squirrels are unlikely to experience such seasonality because they breed throughout the year, not in distinct seasons (Bouchie et al. 2006).

Other than allogrooming, social living may also reduce parasitism via dilution–encounter effects (Mooring & Hart 1992). Larger group sizes may moderate per capita ectoparasite abundance via dilution, where ectoparasites spread out amongst individuals in contact with each other (Mooring & Hart 1992; Johnson et al. 2004). Dilution alone will not change total or average group parasite abundances (Mooring & Hart 1992). Individuals with relatively greater ectoparasite abundances will benefit from the dilution effect by a reduction in infection, while individuals with relatively lower ectoparasite abundances will suffer from increases (Johnson et al. 2004). Furthermore, if larger animal groups are not proportionally more noticeable to parasites or their vectors than solitary animals, termed the encounter effect, then, coupled together, dilution-encounter effects will decrease per capita parasite infections as group sizes increase, since a stable or slowly rising number of parasites will be distributed among a more quickly increasing number of group members (Mooring & Hart 1992). Among alpine marmots (*Marmota marmota*), there was no relationship between group size and ectoparasite abundance, but marmots had fewer ectoparasites per capita in areas with greater marmot clumping (Arnold & Anja 1993). Arnold & Anja (1993) suggest that marmots may seek out areas with fewer ectoparasites, or that marmots clumped in better habitat have better body condition and consequently fewer ectoparasites, but dilution-encounter effects could also offer some explanation. Dilution-encounter effects may also help explain why larger Cape ground squirrel social groups did not have more ectoparasites per capita even though the overall number of parasites in the group did increase with larger group sizes.

We found a positive relationship between affiliative eigenvector centrality and ectoparasite abundance in adult females, but not with agonistic eigenvector centrality. Agonistic interactions in this species are overwhelmingly non-contact and thus unlikely to cause ectoparasite transmission (Chapter 2). Squirrels with more ectoparasites might become more central because they are seeking to reduce their ectoparasite abundance via allogrooming (MacIntosh et al. 2012) or dilution effects (Johnson et al. 2004). Alternatively, more central squirrels may acquire more ectoparasites because they have greater contact with infected individuals (MacIntosh et al. 2012; Rimbach et al. 2015; White et al. 2017).

We found neither affiliative nor agonistic eigenvector centrality correlated with estimates of survival or with estimates of reproductive success. Parasites can be a significant cost of sociality (Johnson et al. 2004; Nunn & Heymann 2005) and this pattern is especially true for ectoparasites among burrowing animals (Butler & Roper 1996). Parasites remove blood and energy, can reduce foraging or vigilance time, and possibly spread diseases or induce a metabolically expensive immune response (Scantlebury et al. 2007). Mothers with fewer calories available can expend less energy on rearing offspring (Scantlebury et al. 2007; Arnold & Anja 1993). Experimentally removing ectoparasites (mainly fleas) from Columbian ground squirrels (*Urocitellus columbianus*) increased female body mass and litter size (Neuhaus 2003). Experimentally removing parasites from Cape ground squirrels greatly increased female reproductive success – probably by increasing the energy and nutrients available to mothers during gestation and lactation, thus increasing infant survival – but did not change the mothers' body mass or litter size (Hillegass et al. 2010). However, this latter study removed both endoparasites and ectoparasites, and the observed reproductive benefit may have been from the removal of the endoparasites, not the ectoparasites, or from removing both together (Hillegass et al. 2010). Parasite fitness costs also may vary with other environmental stressors (Krasnov & Matthee 2010). Our study year had above-average rainfall, and the negative impacts of parasites may be more apparent during years with lower rainfall and thus more overall physiological stress (O'Brien et al. *In Press*).

Or, being more central might have a reproductive benefit that balances in a trade-off with the cost of greater ectoparasite abundance (Duboscq et al. 2016), as centrality in social networks correlates positively to individual longevity and reproductive estimates in a variety of animal taxa, including insects (e.g. forked fungus beetles, *Bolitotherus cornutus*, Formica et al. 2012),

birds (e.g. great tits, *Parus major*, Farine & Sheldon 2015), and mammals (e.g. chacma baboons, *Papio hamadryas ursinus*, Silk et al. 2010, Cheney et al. 2016; Assamese macaques, *Macaca assamensis*, Schülke et al. 2010; horses, *Equus ferus caballus*, Cameron et al. 2009; cats, *Felis catus* L., Natoli et al. 2007; European rabbits, *Oryctolagus cuniculus*, von Holst et al. 2002; brown rats, *Rattus norvegicus domestica*, Yee et al. 2008; yellow-bellied marmots, *Marmota flaviventris*, Wey & Blumstein 2012, but see Blumstein et al. 2018; and bottlenose dolphins, *Tursiops* sp., Stanton & Mann 2012). If centrality has any fitness benefit to adult female Cape ground squirrels, that benefit could explain why more central adult females do not have lower reproductive success despite having more ectoparasites, assuming ectoparasites are costly. Adult female Cape ground squirrels use affiliative interactions, including allogrooming, to reward alloparental care from natal males (LaFlèche et al. *Submitted*). Higher ectoparasite abundance may be a cost of greater centrality from performing allogrooming and other affiliative behaviours by adult females. Higher ectoparasite abundances may also be a cost associated with the benefit of receiving more alloparental care from natal males. In chacma baboons, female eigenvector centrality of mothers predicted their offspring survival better than dyadic (paired) bond strength (Cheney et al. 2016). However, baboon offspring inherit the social dominance ranks of their mothers, and Cape ground squirrel females have no dominance hierarchies (Silk et al. 1999; Waterman 1995). In bottlenose dolphins (*Tursiops* sp.), the eigenvector centrality of male offspring (calves) predicted their survival (Stanton & Mann 2012). Stanton & Mann (2012) suggest that less socially central male calves have greater stress and mortality because they endure more frequent aggression from older juvenile males of their same species. Since Cape ground squirrels are a long-lived species relative to other rodents (living up to 13 years; Zumpt 1970), and since they have unusually low rates of aggression, no observed infanticide, and no female dominance hierarchies (Waterman 1995; Pettitt & Waterman 2011), a study assessing their centrality with survival and offspring survival over a lifetime would also be warranted.

In conclusion, we did not find that per capita ectoparasite abundances increased with larger group sizes in Cape ground squirrels, but we did find that adult females with greater direct and indirect social connection (eigenvector centrality) had higher ectoparasite abundances. Either per-capita ectoparasite abundances did not increase with group size due to dilution-encounter effects, or else Cape ground squirrels mitigated the increasing ectoparasite numbers in larger groups by increasing their grooming behaviour. We did not find that allogrooming rates were

related to group size, but we did not analyze autogrooming behaviour. Our results did not detect a significant fitness cost of having greater eigenvector centrality or ectoparasite abundance. While parasites have been demonstrated as highly costly to adult female fitness in this species (Hillegass et al. 2010), endoparasites may be more costly than ectoparasites (Hillegass et al. 2010), or ectoparasites may only be costly in years with low rainfall (O'Brien et al. *In Press*). Ectoparasite costs may also be matched in a fitness trade-off with some other benefit arising from greater social centrality. Since adult female Cape ground squirrels use allogrooming interactions as a reward for alloparental care from natal males (LaFlèche et al. *Submitted*), one possible benefit of greater social centrality may be greater alloparental care directed towards their offspring. A social network analysis comparing rates of alloparental care with centrality scores would test for such a relationship. Our study shows understanding the costs and benefits of group living in species with low rates of aggression can have novel implications for the relationships between fitness and sociality.

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Tables and figures

Table 3.1: Linear regression results for individual network metrics – affiliative (Aff) and agonistic (Ag) interaction frequencies (Freq) and eigenvector centralities (Centrality) – and fitness estimates – the number of offspring in 2017 (Offspring), the age in 2017 (Age), and survival after 2017 (Survival) – in adult female Cape ground squirrels.

Dependent variable	**Independent variable**	**Coefficient**	**F**	**DF**	**DF**	**Multiple R^2**	***P***
Survival							
	Age	-0.066	0.346	1	24	0.014	0.562
	Offspring	-0.149	0.885	1	24	0.036	0.356
	Aff Freq	-0.298	0.327	1	24	0.013	0.573
	Ag Freq	1.209	1.886	1	24	0.073	0.182
	Aff Centrality	8.65E+15	1.036	1	23	0.043	0.319
	Ag Centrality	1.92E+15	0.139	1	23	0.006	0.713
Offspring							
	Age	0.184	1.760	1	24	0.030	0.197
	Aff Freq	0.151	0.052	1	24	0.002	0.822
	Ag Freq	-0.819	0.512	1	24	0.021	0.481
	Aff Centrality	-6.26E+15	0.280	1	23	1.26E-02	0.602
	Ag Centrality	1.19E+16	4.009	1	23	0.148	0.057
Aff Freq							
	Age	0.081	3.891	1	24	0.140	0.060
Ag Freq							
	Age	0.023	0.831	1	24	0.033	0.371
Aff Centrality							
	Age	-1.98E-18	0.527	1	23	0.022	0.475
Ag Centrality							
	Age	-0.007	1.205	1	24	0.048	0.283

Figure 3.1: Linear representation of the relationship between ectoparasite abundance and affiliative eigenvector centrality among adult female Cape ground squirrels found in a generalized linear mixed model with a negative binomial distribution and social group as a random effect.

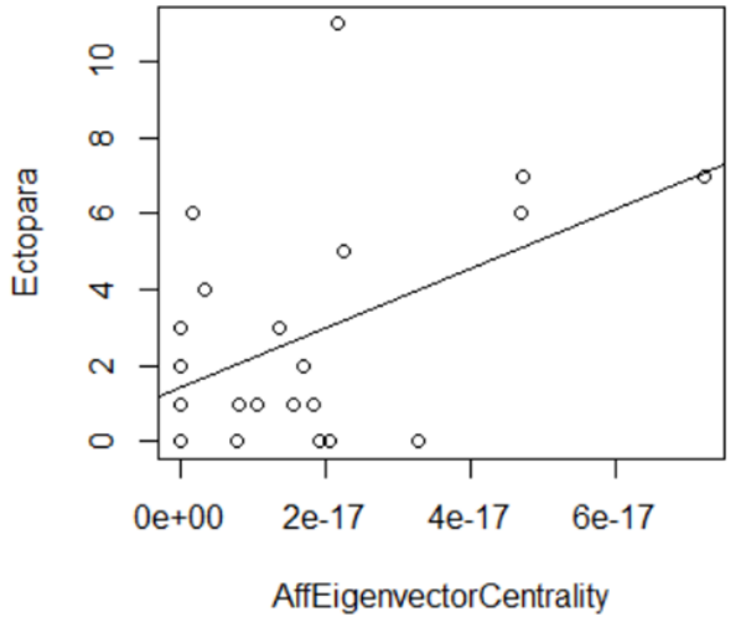
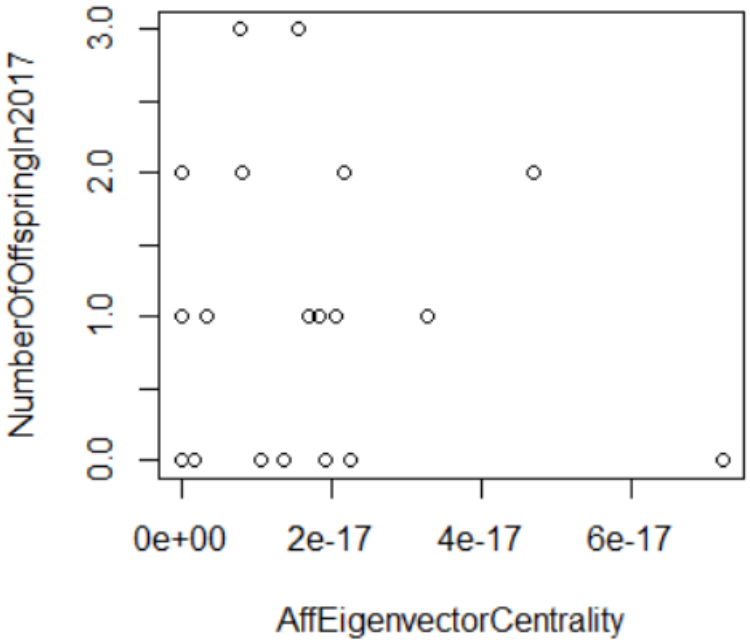


Figure 3.2: No relationship between adult female affiliative eigenvector centrality and the number of their offspring detected in 2017 in Cape ground squirrels.



Appendix

Figure A.3.1: Histogram of the frequency of the number of ectoparasites collected on individual Cape ground squirrels at the time of their first capture.

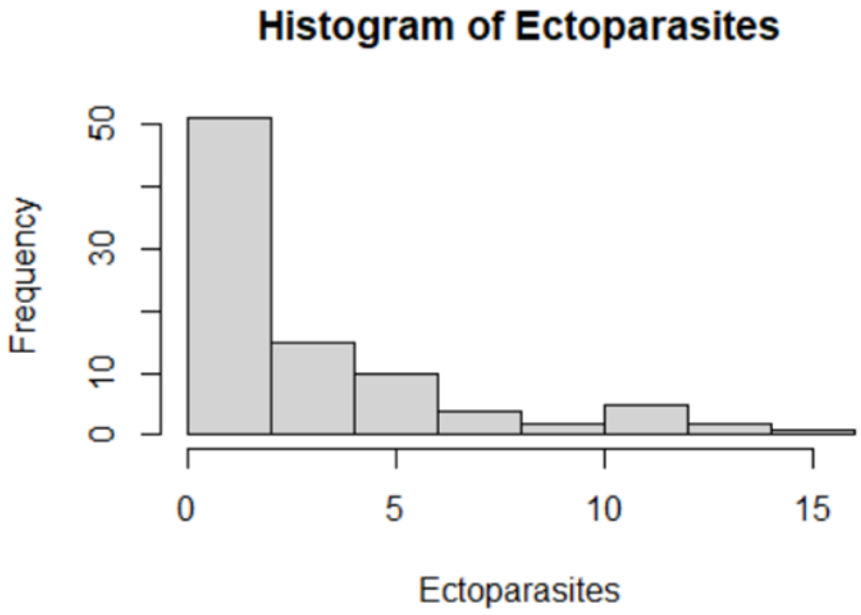


Figure A.3.2: Histogram of the frequency of the transformed eigenvector centrality scores for affiliative interaction frequencies of adult female Cape ground squirrels among their social groups.

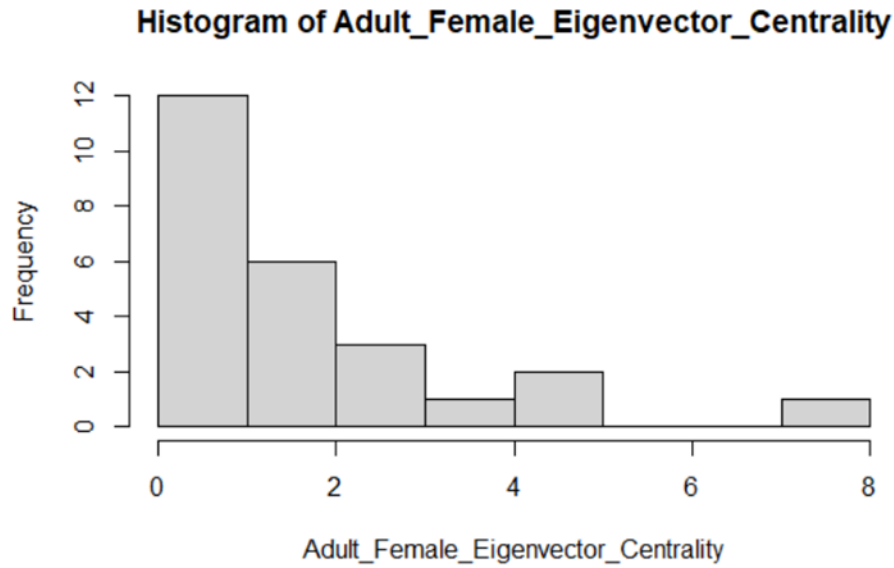


Figure A.3.3: Histogram of the frequency of the rates of allogrooming interactions *received from* another squirrel per observation hour among Cape ground squirrels.

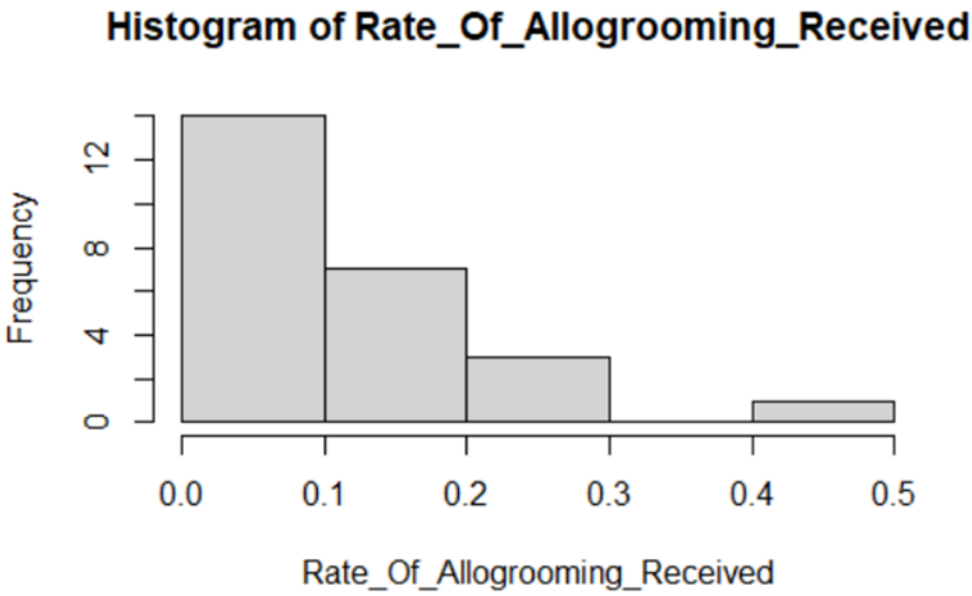


Figure A.3.4: Histogram of the frequency of the rates of allogrooming interactions *given to* another squirrel per observation hour among Cape ground squirrels.

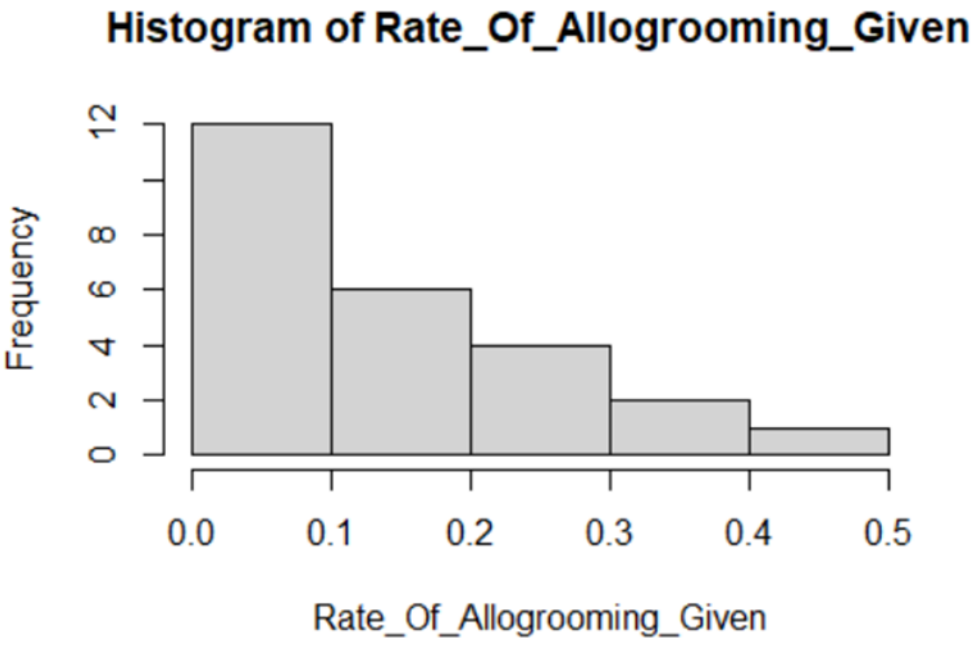


Figure A.3.5: Box-and-whisker plot of the affiliative interaction frequencies among sex-age classes of Cape ground squirrels.

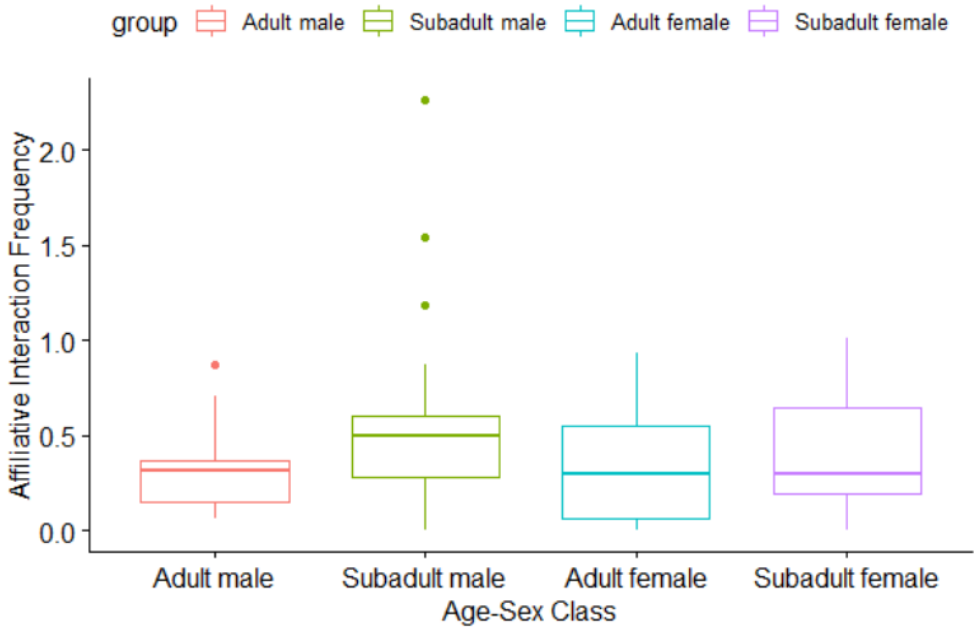
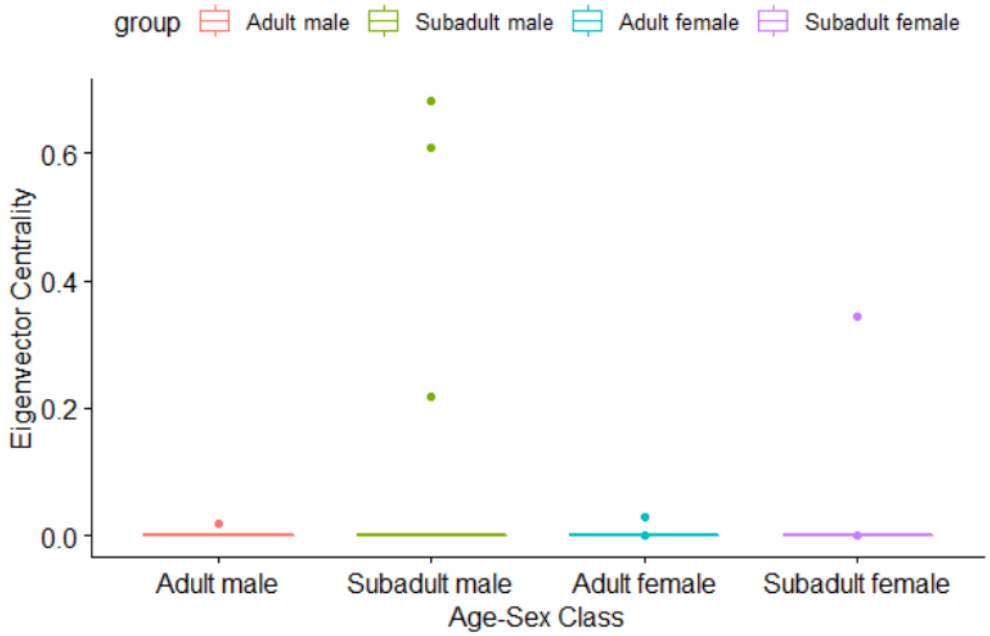


Figure A.3.6: Box-and-whisker plot of the eigenvector centralities of affiliative interaction frequencies among sex-age classes of Cape ground squirrels.



Chapter 4: Thesis conclusions

We found relatedness, sex-homophily, and age-heterophily positively predicted affiliative interaction frequency networks within social groups. Relatedness also positively predicted agonistic interaction frequency networks, and primary associates were not mostly parent-offspring pairs. We found that the average pair-wise relatedness within social groups was greater than expected by chance, confirming that Cape ground squirrel social groups are closely related. We also found that centrality within social interaction networks positively predicted ectoparasite abundance but not estimates of fitness. Interaction frequencies and allogrooming frequencies (received or given) did not predict ectoparasite abundance, and group size did not predict allogrooming rates or per capita ectoparasite abundance.

Our study shows that Cape ground squirrels are nepotistic with their interactions within their social groups. Whether they are nepotistic with squirrels in other social groups remains unknown. A similar analysis as that performed in this study but including interactions amongst social groups could test whether nepotism exists in the interactions among squirrels of different social groups. Most squirrels had extremely low eigenvector centrality scores, while a few squirrels had much higher scores, perhaps because each of the social groups had roughly independent social networks from each other. Our study also shows that the costs and benefits of group living are complex. Centrality within social networks may correlate to fitness benefits beyond what we measured, particularly regarding predation avoidance. Predators of Cape ground squirrels include black-backed jackals (*Canis mesomalas*), snakes (e.g. Cape cobra, *Naja nivea*), raptors (e.g. pale chanting goshawks, *Melierax canorus*), caracals (*Caracal caracal*), brown hyenas (*Hyaena brunnea*), monitor lizards (*Varanus exanthematicus*), and other mesopredators (Unck et al. 2009). The squirrels respond to predators by emitting alarm calls and running to their natal burrow cluster, then either entering a burrow or reaching maximum vigilance posture, alarm calling, and tail-flicking; and they respond to alarm calls similarly (Waterman 2002; Furrer & Manser 2009; Unck et al. 2009; Edwards & Waterman 2011; Waterman & Mai 2020). So, information about approaching predators likely passes among Cape ground squirrels, and squirrels that are more socially central may have faster, more frequent, or more reliable access to information about approaching predators, which may improve their survival rates (Dall et al. 2005; Evans et al. 2020). More central squirrels also may tend to have more squirrels around them, which might increase the chance of detecting predators and reduce the chance of being

targeted by predators for each squirrel (Hamilton 1971; Pulliam 1973; Edwards & Waterman 2011; O'Brien et al. 2018). Cape ground squirrel mothers also actively harass (mob) snakes and monitor lizards – often in unison with other squirrels – to protect their offspring (Waterman 1997; Phillips & Waterman 2014). If greater centrality correlates to more squirrels in proximity (spatially or via information transfer among conspecifics; Evans et al. 2020) available to cooperatively mob predators, then greater centrality might increase the chances of success in the mobbing of snakes and monitor lizards (Waterman 1997; Phillips & Waterman 2014). However, it has not been demonstrated in this species whether behavioural centrality correlates to conspecific responsiveness to predators, or whether the number of mobbing participants predicts mobbing success (Waterman 1997; Phillips & Waterman 2014). Measurements of vigilance behaviour, responsiveness to predators, and conspecific spatial associations or densities could inform social network analyses comparing centrality to predation prevention mechanisms.

Centrality may also correlate to fitness costs that we did not measure, such as contact events that occur at night. Cape ground squirrel social groups share a sleeping burrow every night, which likely provides opportunity for ectoparasite transmission through close direct and indirect contact (Herzig-Straschil 1978; Waterman 1995; Johnson et al. 2004). Leu et al. (2010) found that more central lizards in social networks of asynchronous sleeping refuge-sharing have more ticks; Corner et al. (2003) found that more central brushtail possums in social networks of synchronous den-sharing were more likely to acquire tuberculosis. European badgers share setts (sleeping dens) in which they are regularly re-infested with ectoparasites from each other (Butler & Roper 1996; Johnson et al. 2004). It is not clear how above-ground social centrality relates to below-ground social centrality, or in what timeframe social contact leads to ectoparasite transmission in Cape ground squirrels.

Although we did not detect a relationship between eigenvector centrality and offspring number in Cape ground squirrel adult females in 2017, we may reach different conclusions by increasing the length of the observation period of the females and their offspring (Cheney et al. 2016). Because the consequences of network interactions may not be immediate, and because social behaviour may change over time in individuals and networks, a longer-term study may yield different results (Wilson et al. 2013; Ilany et al. 2015). Social network position depends on both an individual's behaviour and the behaviour of the other individuals in the social group, so social networks may be considered the extended phenotype of social behaviours and may change

over time in response to natural selection acting on heritable phenotypes, or to changes in group composition, or to changes in individual behaviour over their lifetimes (Dawkins 1982; Formica et al. 2012; Wilson et al. 2013; Ilany et al. 2015). However, if network metrics are consistent over time, or even if the relationships between network metrics are consistent over time, then we can expect no change in our results even given a longer study period (Wilson et al. 2013). Whether network metrics change over time has itself not yet been studied in Cape ground squirrels. We have reason to suspect Cape ground squirrel networks change over time because social groups split once they grow large and because individuals disappear, presumably because of predation (Waterman 2002). Potential changes over time may also affect ectoparasite abundances and their fitness consequences, if ectoparasites have fitness costs that are not immediate (Arnold & Anja 1993). Also, our estimate of ectoparasites may not be representative of individuals' actual typical ectoparasite abundance. We estimated ectoparasites by the number of ectoparasites recovered during each squirrel's first capture and handling, which included standardized flea combing and thigh and groin inspection (Scantlebury et al. 2007; Hillegass et al. 2008; Beaumont et al. 2019). The same method used on the same species produced ectoparasite abundance estimates that correlated to the total number of ectoparasites thoroughly collected on sacrificed squirrels (Beaumont et al. 2019). However, an average number of ectoparasites over a longer period might be more representative of individuals' typical ectoparasite abundance, or individual ectoparasite abundances may fluctuate over time (Krasnov et al. 2006).

Allogrooming ectoparasites is likely a mutually beneficial behaviour that can mitigate increasing ectoparasite abundance as one of the costs of social interactions (Hart 1990; Kingma et al. 2014). Allogrooming may be sought out as a benefit of social living, or as mitigation for a cost of social living, and is a classic example of mutualistic behaviour (Hart 1990; Johnson et al. 2004). Conspecifics often exchange allogrooming services in a short time span, an example of direct fitness benefits from social interactions (Kingma et al. 2014). Fleas are highly mobile and can jump on and off squirrels; they do not require social interactions to spread but are likely to spread more with more contact interactions (Johnson et al. 2004; Krasnov & Matthee 2010). Lice are not as mobile and likely require contact to spread (Duboscq et al. 2016). Ticks are rare on Cape ground squirrels in South Africa (Beaumont et al. 2019), unlike in Namibia (Waterman 2002). The consequences of allogrooming might depend on the type of ectoparasite (Krasnov &

Matthee 2010). Allogrooming might be more effective at reducing lice abundances than reducing flea abundances, because fleas are more mobile (Hart 1990; Johnson et al. 2004; Krasnov & Matthee 2010; Duboscq et al. 2016).

The relationship between allogrooming and ectoparasites is complex for three reasons: 1) allogrooming rates and ectoparasite abundances may cycle with each other. Allogrooming in this species is a response to ectoparasites and is likely effective at removing ectoparasites (Hillegass et al. 2010; and see Hart 1990, Johnson et al. 2010, Duboscq et al. 2016). As ectoparasites increase, allogrooming may increase, and as allogrooming increases, ectoparasites may decrease, and so on. Measuring the relationship between the two may require temporal sampling (e.g. Krasnov et al. 2006). Changes in ectoparasite abundance and allogrooming rates may change on relatively rapid time scales that a single ectoparasite sampling event would not detect (Hillegass 2010; Krasnov et al. 2006). 2) Every allogrooming interaction is also a close contact interaction that has the potential to transmit ectoparasites between both interacting squirrels (Duboscq et al. 2016). Contact interactions are undirected; allogrooming is directed (Rimbach et al. 2015; Duboscq et al. 2016). However, contact between squirrels with different numbers of ectoparasites may have a directional average transfer of ectoparasites towards the squirrel with fewer ectoparasites (Johnson et al. 2004). A directed ectoparasite transfer depends on the dilution effect of ectoparasite transmission from social contact applying, and it is not clear whether that equitable dilution effect does apply in this study system (Hillegass et al. 2008; Johnson et al. 2004; Krasnov et al. 2006; Silk et al. 2018). Furthermore, since squirrels with more ectoparasites also may be more likely to be allogroomed (Hillegass et al. 2010), this directional transfer effect predicts that giving allogrooming may increase ectoparasite abundance and reinforces the prediction that receiving allogrooming may decrease ectoparasite abundance (Rimbach et al. 2015). 3) If ectoparasites readily spread out amongst social group members, then reducing ectoparasites on any squirrel in the social group may, in effect, decrease them on every squirrel in the social group (Johnson et al. 2004). Allogrooming may therefore decrease the ectoparasite abundance on both the allogrooming receiver and the allogrooming giver, given enough time for ectoparasites to diffuse through the group (Johnson et al. 2004). This dilution effect of ectoparasites and allogrooming may favour the evolution of seemingly altruistic behaviour (Johnson et al. 2004). If it benefits each member of a group to reduce the total number of ectoparasites in the group, individuals may be incentivized to allogroom others for their own

benefit or the benefit of their own offspring – even in the absence of reciprocity (Johnson et al. 2004). Additionally, if an increasing group size overwhelms the capacity of allogrooming to mitigate increasing ectoparasite abundance, group splitting may then be favoured (Johnson et al. 2004). Behavioural responses – like grooming and group splitting – to increasing ectoparasite abundance in larger social groups may help explain why ectoparasites often do not correlate to group sizes, and why groups may fission after growing larger, and why group sizes remain stable over time (Dunbar 1991; Johnson et al. 2004; Lucatelli et al. 2020).

Dilution effects may be more complex if ectoparasites preferentially conglomerate on particular individuals, due to, for instance, differences in host allogrooming rates, body condition, hormones, or immune response (Johnson et al. 2004; Muehlenbein & Bribiescas 2005; Hillegass et al. 2008). In Cape ground squirrels, ectoparasites are, on average, more abundant on males; and females, on average, perform more allogrooming (Hillegass et al. 2008). Adding ectoparasite abundance to a future social network ERGM could test for influences from such effects (Silk et al. 2018).

Mechanistic evolutionary analysis attempts to associate phenotypes with their fitness consequences (Grant & Grant 2000; Silk 2007; Formica et al. 2012; Farine & Sheldon 2015). Fitness consequences can be costs or benefits, and they can be direct or indirect, where direct refers to the reproductive success of an organism and its descendants, and indirect refers to the effects an organism's phenotype has on the reproductive success of its close relatives (Covas & Doutrelant 2019; Boomsma 2013; Smith 2014). Direct and indirect fitness benefits are not mutually exclusive (Hamilton 1964). Kinship may provide a basis for interaction associations that increase the reliability of mutualism or reciprocity (Armitage 1981; Clutton-Brock 2009; Kingma et al. 2014). The direct benefits of mutual or reciprocal behaviour may be insufficient to support helping behaviour among non-kin, and, conversely, the inclusive benefits of kinship alone may be insufficient to support a costly behaviour without the reward of reciprocity (Hamilton 1964; Maynard Smith 1964). Fitness itself is a poorly defined concept, arguably more applicable to alleles than to organisms (Dawkins 1982). In our study, we used fitness as a metric seeking to estimate individual evolutionary success. One problem with defining fitness is that fitness measurements should ideally extend perpetually (Dawkins 1982). An organism that has many children but no grandchildren may be said to have zero fitness, for instance. So, our estimates of fitness are necessarily limited by time. Survival is not important for fitness except

that survival is necessary to reproduce, and that makes survival crucial for fitness. An organism that lives extremely long but never reproduces has zero fitness, the same as an organism that dies young before it can reproduce. Survival and reproductive success – the metrics we used – and the survival of offspring are all estimates attempting to approximate fitness. Lifetime reproductive success and offspring reproductive success across multiple generations down family trees is an increasingly better approximation of fitness. However, the longer the length of time included, the increasingly less confident we can be in correlating momentary behavioural differences with long-term fitness consequences. Comparing a figurative snapshot of an organism's behaviour to a snapshot of its fitness outcomes might not just be more feasible in a study; it might also be more temporally appropriate. Understanding whether and how behaviour changes over a lifetime, how the rates of reproduction ordinarily fluctuate over a lifetime, and to what degree behaviours are heritable, together those all inform evolutionary analyses of the fitness consequences of behaviour, and they are beyond the scope of this study.

In conclusion, this thesis addresses knowledge gaps in describing Cape ground squirrel social complexity in terms of their social networks. We had hypothesized that Cape ground squirrels would show behavioural kin biases due to inclusive fitness benefits, predicting that they will interact affiliatively more often and agonistically less often with closer kin within their social groups (Van Belle et al. 2014). We found support for the former but not the latter prediction. Since agonistic interactions are relatively mild in this species, greater agonistic interaction frequencies with closer kin may have insignificant fitness costs (Viblanc et al. 2016). We detected sex biases that may be due to direct and indirect fitness benefits associated with predator avoidance and natal philopatry, and we detected age biases that may be due to direct fitness benefits associated with pay-to-stay alloparental behaviour (LaFlèche et al. *Submitted*). This thesis also highlights evolutionary implications for the relative positions of Cape ground squirrels in their social networks, particularly as their social interactions relate to ectoparasite transmission (Brent 2015). Centrality within affiliative interaction networks predicted ectoparasite abundance among adult female Cape ground squirrels. Squirrels with more ectoparasites might become more central because they are seeking to reduce their ectoparasite abundance via allogrooming (MacIntosh et al. 2012) or dilution effects (Johnson et al. 2004). Alternatively, more central squirrels may acquire more ectoparasites because they have greater contact with infected individuals (MacIntosh et al. 2012; Rimbach et al. 2015; White et al. 2017).

Cape ground squirrels are unusual in their lack of matrilineal dominance hierarchies and in the low rates and mild consequences of their agonistic interactions (Waterman 1995, 1996; this study; Viblanc et al. 2016). This unusual social system allows better detection of other social factors with fitness consequences that are normally obscured by aggression, such as grooming behaviour serving simply to remove ectoparasites, instead of as an expression of dominance or submission (Viblanc et al. 2016; Hillegass et al. 2010; Schino 2001; Madden & Clutton-Brock 2009). This thesis supports the findings of the meta-analysis on the relationship between sociality and parasitism across mammalian taxa: that social structure predicts parasitism, and group size does not predict parasitism (Lucatelli et al. 2020). Larger groups might not suffer greater per capita ectoparasite abundances because of encounter-dilution effects spreading out a limited number of parasites among more group members, or because group members change their behaviour as group sizes increase (Mooring & Hart 1992; Kutsukake & Clutton-Brock 2006; Johnson et al. 2004). Group members could groom more or split their groups as group sizes become too large (Kutsukake & Clutton-Brock 2006; Johnson et al. 2004). We found that group size did not predict allogrooming frequencies, but that result could be an artifact of allogrooming being both 1) effective at reducing ectoparasites and 2) quickly responsive to lower ectoparasite abundances. Autogrooming may also increase instead of allogrooming (Hawlena et al. 2007). To detect if group splitting is effective at reducing ectoparasite abundances, we need to analyze ectoparasite abundances in groups before and immediately after they split. Next, we did not detect a significant fitness cost of having greater eigenvector centrality or greater ectoparasite abundance. While parasites have been demonstrated as highly costly to adult female fitness in this species (Hillegass et al. 2010), endoparasites may be more costly than ectoparasites (Hillegass et al. 2010), or ectoparasites may only be costly in years with low rainfall (O'Brien et al. *In Press*). Ectoparasite costs may also be matched in a fitness trade-off with some other benefit arising from greater social centrality (Duboscq et al. 2016). Since adult female Cape ground squirrels use allogrooming interactions as a reward for alloparental care from natal males (LaFlèche et al. *Submitted*), one possible benefit of greater social centrality may be greater alloparental care directed towards their offspring; another possible fitness benefit of social centrality is more information about detecting approaching predators (Hamilton 1971); and another is having more conspecifics around to dilute or defend from predation (Pulliam 1973; Waterman 1997). Finally, our results address the more general knowledge gaps of whether

promiscuous cooperative breeders must be nepotistic (Cornwallis et al. 2010), whether social network metrics can predict parasitism (Lucatelli et al. 2020), and whether social network metrics – even in the absence of aggression and dominance hierarchies – can predict fitness estimates (Viblanc et al. 2016; Krause et al. 2015).

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