Intra and intergroup patterns of relatedness and space use in the cooperative and promiscuous breeding Cape ground squirrel (*Xerus inauris*)

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Abstract

Group living is an important life-history tactic for many species and provides several benefits, such as predation protection and cooperative breeding, but can also incur costs. Ultimately, group living may evolve in a species if its benefits outweigh its costs. When groups become large, these costs can increase and can be detrimental for the reproductive success of gregarious females. Kin selection is one mechanism that can offset group costs and is considered the primary selective force leading to cooperative breeding in mammals. Hypotheses on the evolution of cooperative breeding predict these groups to be monogamous to maintain high relatedness within their family groups. In the case of promiscuous cooperative breeding species, relatedness may be highly variable both within and among groups, but little is known about if and how kin selection functions in these groups. Cape ground squirrels (*Xerus inauris*) are a cooperative breeding species with a promiscuous breeding system. Our objective was to first characterize the importance of kin selection within groups by examining relatedness within Cape ground squirrel family groups, if kin bias was important during foraging away from safety, and if kin selection was an important factor when Cape ground squirrel females dispersed from their family group. Secondly, we characterized relatedness patterns among family groups across a landscape and determined if there was a kin bias or benefit to the sharing of spatial resources among family groups. We collected observational data on 15 different squirrel family groups in central South Africa. Our results indicated that promiscuity influenced relatedness and group size, and that, over time, relatedness declined for males but not for females in a group. When females left their group, they usually did so with their own offspring, although we found two fissions that included females splitting with close kin that were not their offspring. When foraging, females did not show a spatial bias towards kin. We found that across a landscape, relatedness decreased with increasing distance, but we did not find that Cape ground squirrels showed a bias in spatial resources between kin, or that spatial resources contributed to reproductive success or survival of females. Overall, female Cape ground squirrels optimized direct fitness benefits when dispersing from their family groups, and overt kin biases were not apparent during foraging. Our study is important in understanding the evolution of cooperative breeding in a promiscuous species with a unique, tolerant, and non-aggressive social system.

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Chapter 1: Thesis Introduction

Group living can have many benefits such as predation protection (via the dilution effect and the many eyes hypothesis; Wrona and Dixon, 1991), resource acquisition, territory defence, and cooperative breeding (Hamilton, 1971). Grouping is common among many taxa including birds, fish, mammals, and insects (Clutton-Brock, 2002; Seppä *et al.*, 2008; McDonald et al., 2016). The costs of living in larger groups include increased resource competition, increased parasite and disease transition, and reproductive suppression (Hamilton, 1971), which often leads to decreased reproductive success for gregarious females (Dunbar, 2018). As a consequence, animals must mitigate costs and maximize the benefits when living in groups (Armitage, 1981).

Kin selection is one way by which animals may mitigate the costs of living in large groups (Smith, 2014). Kin selection operates through inclusive fitness, where an individual will favour altruistic behaviour towards more closely related individuals because they share more genes. By biasing altruistic behaviour towards closer kin, individuals may propagate shared genetic information into the next generation (Hamilton, 1964; Smith, 2014). Across taxa, many species demonstrate kin biases in their behaviour (e.g., paper wasps (*Polistes dominula*), cichlid fish (*Neolamprologus pulcher*), chestnut-crowned babbler (*Pomatostomus ruficep*), African lions, spotted hyenas (*Crocuta crocuta*), and gelada baboons (*Theropithecus gelada*) (John *et al.*, 2019; Hellmann *et al.*, 2016; Browning *et al.*, 2012; Packer *et al.*, 1991;Van Horn *et al.*, 2004; Dunbar, 2018).

In mammals, females are usually the philopatric sex, and female groups, or matrilines, form the basis for the structure and subsequent social interactions of many types of mammalian societies (Lukas et al., 2005; Mattison et al., 2019). In many cooperative breeding groups, breeding is limited to a single breeding pair, and the resulting matrilines are made up of close kin (Clutton-Brock and Lukas, 2012). Some studies have identified such lifetime monogamy as essential for the evolution of cooperative breeding, as relatedness among offspring would remain high, ensuring inclusive fitness benefits to these helpers (Lukas and Clutton-Brock, 2012). In comparison, in promiscuous mating systems, relatedness among group members can vary greatly and group relatedness is predicted to decrease with increasing group size and over generations (Aviles et al., 2004; Lukas et al., 2005). However, kin discrimination in groups where there is a wide variance in relatedness can still result in inclusive fitness for cooperative breeders despite the promiscuous breeding system (Cornwallis *et al.*, 2010; Kramer and Russell, 2014). Individuals could direct helpful behaviour towards kin, if they are capable of kin discrimination (Cornwallis et al., 2010; Kramer and Russell, 2014). There are several mechanisms by which individuals may discriminate kin from non-kin, such as spatial dispersal, prior familiarity, the green beard effect, and phenotype matching (Mateo, 2003). More specifically, prior association is when individuals learn who they are related to in early development and later will discriminate between familiar and unfamiliar individuals regardless of actual genetic relatedness (Mateo, 2003). Phenotype matching is where individuals discriminate between related and non-related individuals based on chemical cues such as odour (Armitage, 1989; Mateo, 2003). Promiscuous species that can discriminate kin on a fine degree of relatedness may even form sub-groups based on kinship during activities like foraging (Hatchwell, 2010; Best *et al.*, 2013; Kramer & Russell, 2014).

Sometimes groups become too large and the costs, such as resource competition, may outweigh the benefits of kin selection, especially if kin compete against one another (Chepko-Sade and Sade, 1979). Dispersal is an important mechanism to avoid the costs of large groups or low kinship (Clutton-Brock and Lukas, 2012). There are several reasons for females to disperse, and these may include decreasing competition with group members and kin, avoiding infanticide, and inbreeding avoidance (Clutton-Brock and Lukas, 2012). Through group splitting, females can form a new smaller group with kin to better balance the costs and benefits of group living (Chepko-Sade and Sade, 1979).

After a group fission, new groups will often settle spatially close to their original group (Wright, 1943). This pattern is known as the isolation by distance pattern, as groups spatially close to one another are more likely to be more related (Wright, 1943). If animals avoid costs of living in a larger group through group splitting but remain spatially close in a new smaller group, there may be intergroup advantages without the consequences of living in larger groups (Aviles et al., 2004). Kin selection can lead to increased longevity and reproduction in some animals (Silk et al., 2003; Silk et al., 2010), and the benefits of nepotistic behaviour may continue after individuals leave the group. For female baboons, social bonds between pairs of females are important for longevity (Silk et al., 2010). In contrast, Black-tailed prairie dogs (*Cynomys ludovicianus*) become aggressive to individuals not in the same group as them, even if they were once in the same group. Space use can be quantified using home range, which can be used as a proxy for resources an organism needs to survive, and the spatial tolerance of animals towards their relatives is often used as a proxy for kin bias (Charif et al., 2005; King, 1989; Maher, 2009; Podgórski, Lusseau et al., 2014; Stoen et al., 2005; Vuren and Armitage, 1994). Kinship may influence where individuals settle if group splitting occurs or how groups share spatial resources (Armitage et al., 2011; Broquet et al., 2006). The analysis of spatial resources, such as individual and group home ranges and proximities, may be helpful in detecting kin biases, especially when behavioural interactions between animals are rare (Lukas and Clutton-Brock, 2018).

Cape ground squirrels (*Xerus inauris)* are a social species that live in distinct kin or family groups in southern Africa (Waterman, 1995). Female matrilines consist of 1-6 adults that form a single family group with their offspring and related subadults of both sexes (Waterman, 1995). Female matrilines are distinct from one another, as members of the family group share a single sleeping burrow at night and communal foraging range in the day (Waterman, 1995; Waterman, 2002). Males disperse and sometimes form all-male bands (hereafter known as band males), but some males may delay dispersal and remain in their natal group (hereafter known as natal males; Waterman, 1998). Females mate with multiple males during a short estrus and have small litters (1-2 offspring). Band males rove around a large home range (encompassing up to 30 family groups) searching for receptive females. Both competitive searching and sperm competition are thought to influence paternity, and 70% of litters of two are multiply sired (Manjerovic and Waterman, 2015; Waterman, 1996). Thus multiple mating by both males and females contributes to a mosaic of relatedness both within and among family groups of this species (Waterman, 1995; Waterman, 1998). Young females may experience reproductive suppression via delayed maturity, and matrilines may split due to this suppression and resource competition (Pettitt et al., 2008; Waterman, 2002). Even though Cape ground squirrels are highly promiscuous, they are also facultative cooperative breeders with alloparental care, or the care of offspring by non-parents (Waterman, 1995; Pettitt and Waterman, 2011). Cape ground squirrels are capable of detecting varying degrees of relatedness through olfaction in kin they have never met, but they do not appear to show such discrimination within their own family groups (Waterman and Archibald, 2019). It is unknown whether Cape ground squirrels show a kin bias through spatial resources, or if kin bias has an influence in group splitting and spatial patterns both within and among groups.

This thesis is divided into two main parts: part one examines within-group relatedness and kinship and how relatedness within a group changes with increasing group size and over time. It also focuses on how relatedness within a group influences group splitting. Geographically, we analyze where groups settle relative to one another and how geographically close individuals are positioned near kin when feeding throughout the day. Part two explores relatedness among groups across the landscape and if home range overlap reflects kin biases among groups in Cape ground squirrels.

References

Armitage, K. B. (1981). Sociality as a life-history tactic of ground squirrels. *Oecologia*, 48, 36–49.

Armitage, K. B. (1989). The function of kin discrimination. *Ethology Ecology & Evolution*, 1, 111–121.

Armitage, K. B., Vuren, D. H. Van, Ozgul, A., & Oli, M. K. (2011). Proximate causes of natal dispersal in female Yellow-bellied marmots, Marmota flaviventris. *Ecology*, 92(1), 218–227.

Aviles, L., Fletcher, J. A., & Cutter, A. D. (2004). The kin composition of social groups: trading group size for degree of altruism. *The American Naturalist*, 164(2), 132–144.

Best, E. C., Seddon, J. M., Dwyer, R. G., & Goldizen, A. W. (2013). Social preference influences female community structure in a population of wild Eastern grey kangaroos. *Animal Behaviour*, 86(5), 1031–1040. https://doi.org/10.1016/j.anbehav.2013.09.008

Broquet, T., Ray, N., Petit, E., Fryxell, J. M., & Burel, F. (2006). Genetic isolation by distance and landscape connectivity in the American marten (Martes americana). *Landscape Ecology*, 21(6), 877–889. https://doi.org/10.1007/s10980-005-5956-y

Browning, L. E., Patrick, S. C., Rollins, L. A., Griffith, S. C., & Russell, A. F. (2012). Kin selection, not group augmentation, predicts helping in an obligate cooperatively breeding bird. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1743), 3861–3869. https://doi.org/10.1098/rspb.2012.1080

Charif, R. A., Ramey, R. R., Langbauer, W. R., Payne, K. B., Martin, R. B., & Brown, L. M. (2005). Spatial relationships and matrilineal kinship in African savanna elephant (Loxodonta africana) clans. *Behavioral Ecology and Sociobiology*, *57*(4), 327–338. https://doi.org/10.1007/s00265-004-0867-5

Chepko-Sade, B. D., & Sade, D. S. (1979). Patterns of group splitting within matrilineal kinship groups: a study of social group structure in Macaca mulatta (Cercopithecidae: Primates). *Behavioural Ecology and Sociobiology*, 5(1), 67–86. Retrieved from https://www.jstor.org/stable/4599218

Clutton-Brock, T. H. (2002). Breeding together: kin selection and mutualism in cooperative vertebrates. *Science*, *296*(5565), 69–72. https://doi.org/10.1126/science.296.5565.69

Lukas, D., & Clutton-Brock, T. H. (2012). Life histories and the evolution of cooperative breeding in mammals. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1744), 4065–4070. https://doi.org/10.1098/rspb.2012.1433

Lukas, D., & Clutton-Brock, T. H. (2012). Life histories and the evolution of cooperative breeding in mammals. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1744), 4065–4070. https://doi.org/10.1098/rspb.2012.1433

Dunbar, R. I. M. (2018). Social structure as a strategy to mitigate the costs of group living: a comparison of gelada and guereza monkeys. *Animal Behaviour*, *136*, 53–64. https://doi.org/10.1016/j.anbehav.2017.12.005

Hamilton, W. D. (1964). The genetical evolution of social behaviour. II. *Journal of Theoretical Biology*, *7*(1), 17–52. https://doi.org/10.1016/0022-5193(64)90039-6

Hamilton, W. D. (1971). Geometry for the selfish herd. *Journal of Theoretical Biology*, 31, 295–311.

Hatchwell, B. J. (2010). Cryptic kin selection: kin structure in vertebrate populations and opportunities for kin-directed cooperation. *Ethology*, *116*(3), 203–216. https://doi.org/10.1111/j.1439-0310.2009.01732.x

Hellmann, J. K., Sovic, M. G., Gibbs, H. L., Reddon, A. R., Connor, C. M. O., Ligocki, I. Y., & Marsh-rollo, S. (2016). Within-group relatedness is correlated with colony-level social structure and reproductive sharing in a social fish. *Molecular Ecology*, 25, 4001–4013. https://doi.org/10.1111/mec.13728

Parsons, P. J., Grinsted, L., & Field, J. (2019). Partner choice correlates with fine scale kin structuring in the paper wasp Polistes dominula. *PLOS ONE*, *14*(8), e0221701. https://doi.org/10.1371/journal.pone.0221701

King, W. J. (1989). Spacing of female kin in Columbian ground squirrels (Spermophilus columbianus). *Canadian Journal of Zoology*, *67*(1), 91–95. https://doi.org/10.1139/z89-014

Kramer, K. L., & Russell, A. F. (2014). Kin-selected cooperation without lifetime monogamy: human insights and animal implications. *Trends in Ecology & Evolution*, *29*(11), 600–606. https://doi.org/10.1016/j.tree.2014.09.001

Lukas, D., & Clutton-Brock, T. (2018). Social complexity and kinship in animal societies. *Ecology Letters*, *21*(8), 1129–1134. https://doi.org/10.1111/ele.13079

Lukas, D, Reynolds, V., Boesch, C., & Viglilant, L. (2005). To what extent does living in a group mean living with kin? *Molecular Ecology*, 14, 2181–2196. https://doi.org/10.1111/j.1365-294X.2005.02560.x

Lukas, D., & Clutton-Brock, T. (2012). Cooperative breeding and monogamy in mammalian societies. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1736), 2151–2156. https://doi.org/10.1098/rspb.2011.2468

Maher, C. R. (2009). Genetic relatedness and space use in a behaviorally flexible species of marmot, the woodchuck (Marmota monax). *Behavioral Ecology and Sociobiology*, *63*(6), 857–868. https://doi.org/10.1007/s00265-009-0726-5

Manjerovic, M. B., & Waterman, J. M. (2015). “Failure to launch”: is there a reproductive cost to males living at home? *Journal of Mammalogy*, *96*(1), 144–150. https://doi.org/10.1093/jmammal/gyu015

Mateo, J. M. (2003). Kin recognition in ground squirrels and other rodents. *Journal of Mammalogy*, 84(4), 1163–1181. https://doi.org/10.1644/ble-011

Mattison, S. M., Shenk, M. K., Thompson, M. E., Borgerhoff Mulder, M., & Fortunato, L. (2019). The evolution of female-biased kinship in humans and other mammals. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*(1780), 20190007. https://doi.org/10.1098/rstb.2019.0007

McDonald, P. G., Rollins, L. A., & Godfrey, S. (2016). The relative importance of spatial proximity, kin selection and potential ‘greenbeard’ signals on provisioning behaviour among helpers in a cooperative bird. *Behavioural Ecology and Sociobiology*, 70, 133–143. https://doi.org/10.1007/s00265-015-2032-8

Packer, C., Gilbert, D. A., Pusey, A. E., & O’Brieni, S. J. (1991). A molecular genetic analysis of kinship and cooperation in African lions. *Nature*, *351*(6327), 562–565. https://doi.org/10.1038/351562a0

Pettitt, B. A., & Waterman, J. M. (2011). Reproductive delay in the female Cape ground squirrel (Xerus inauris). *Journal of Mammalogy*, *92*(2), 378–386. https://doi.org/10.1644/10-MAMM-A-168.1

Pettitt, B. A., Waterman, J. M., & Wheaton, C. J. (2008). Assessing the effects of resource availability and parity on reproduction in female Cape ground squirrels: resources do not matter. *Journal of Zoology*, *276*(3), 291–298. https://doi.org/10.1111/j.1469-7998.2008.00491.x

Podgórski, T., Lusseau, D., Scandura, M., Sönnichsen, L., & Jędrzejewska, B. (2014). Long-lasting, kin-directed female interactions in a spatially structured wild boar social network. *PLoS ONE*, *9*(6), e99875. https://doi.org/10.1371/journal.pone.0099875

Seppä, P., Fernández-Escudero, I., Gyllenstrand, N., & Pamilo, P. (2008). Colony fission affects kinship in a social insect. *Behavioral Ecology and Sociobiology*, *62*(4), 589–597. https://doi.org/10.1007/s00265-007-0483-2

Silk, J. B. (2003). Social Bonds of Female Baboons Enhance Infant Survival. *Science*, *302*(5648), 1231–1234. https://doi.org/10.1126/science.1088580

Silk, J. B., Beehner, J. C., Bergman, T. J., Crockford, C., Engh, A. L., Moscovice, L. R., … Cheney, D. L. (2010). Strong and consistent social bonds enhance the longevity of female baboons. *Current Biology*, *20*(15), 1359–1361. https://doi.org/10.1016/j.cub.2010.05.067

Smith, J. E. (2014). Hamilton's legacy: kinship, cooperation and social tolerance in mammalian groups. *Animal Behaviour*, *92*, 291–304. https://doi.org/10.1016/j.anbehav.2014.02.029

Støen, O.-G., Bellemain, E., Sæbø, S., & Swenson, J. E. (2005). Kin-related spatial structure in brown bears Ursus arctos. *Behavioral Ecology and Sociobiology*, *59*(2), 191–197. https://doi.org/10.1007/s00265-005-0024-9

Van Horn, R. C., Engh, A. L., Scribner, K. T., Funk, S. M., & Holekamp, K. E. (2004). Behavioural structuring of relatedness in the spotted hyena (Crocuta crocuta) suggests direct fitness benefits of clan-level cooperation. *Molecular Ecology*, *13*(2), 449–458. https://doi.org/10.1046/j.1365-294X.2003.02071.x

Van Vuren, D., & Armitage, K. B. (1994). Reproductive success of colonial and noncolonial female Yellow-bellied marmots (Marmota flaviventris). *Journal of Mammalogy*, *75*(4), 950–955. https://doi.org/10.2307/1382476

Waterman, J. M. (1995). The social organization of the cape ground squirrel (Xerus inauris; Rodentia: Sciuridae). *Ethology*, *101*(2), 130–147. https://doi.org/10.1111/j.1439-0310.1995.tb00352.x

Waterman, J. M. (1998). Mating tactics of male Cape ground squirrels,Xerus inauris: consequences of year-round breeding. *Animal Behaviour*, *56*(2), 459–466. https://doi.org/10.1006/anbe.1998.0780

Waterman, J. M. (2002). Delayed maturity, group fission and the limits of group size in female Cape ground squirrels (Sciuridae: Xerus inauris). *Journal of Zoology*, *256*(1), 113–120. https://doi.org/10.1017/S0952836902000146

Waterman, J. M. (1996). Reproductive biology of a tropical, non-hibernating ground squirrel. *Journal of Mammalogy*, *77*(1), 134–146. https://doi.org/10.2307/1382715

Waterman, J. M., & Archibald, A. J. (2019). Both familiarity and kinship influence odour discrimination by females in a highly social African ground squirrel. *Animal Behaviour*, *148*, 145–151. https://doi.org/10.1016/j.anbehav.2018.12.007

Wright, S. (1943). Isolation by distance. *Genetics*, 28(2), 114–138. https://doi.org/Article

Wrona, F. J., & Jamieson Dixon, R. W. (1991). Group size and predation risk: A field analysis of encounter and dilution effects. *The American Naturalist*, *137*(2), 186–201. https://doi.org/10.1086/285153

Chapter 2: Intragroup spatial relations and kinship in Cape ground squirrels

Abstract

Animals may live in groups for many benefits, such as enhanced predator detection and cooperative breeding. However, there are also costs to group living, like resource competition and reproductive suppression. In cooperative breeding species, kin selection may offset costs by group members favouring closely related members due to the higher possibility they share genes. Some mammals exhibit female philopatry where females remain in the area they were born and may form kin groups called matrilines. However, in promiscuous breeding species, relatedness is predicted to decrease with increasing group size. Despite varying relatedness within groups, some mammals can discriminate kin on a fine-scale and can form sub-groupings in which to direct kin biased behaviour. Cape ground squirrels are a promiscuous, cooperative breeding species that lives in female matrilines. We hypothesized that if promiscuous breeding affects group relatedness, then relatedness would decline over time and decline with increasing group size. Further, if inclusive fitness is important to Cape ground squirrels, groups will split along kin lines, and individuals will maintain close spatial proximity to more closely related individuals when foraging. Our study was conducted on S.A. Lombard Nature Reserve in South Africa. We observed 15 different family groups using scan sampling and used our long-term dataset for our analysis on matrilines over time. We have found that promiscuity affected overall group relatedness, and group relatedness declined with group size and time. However, when we only examined females in the group, matriline relatedness declined in larger groups but did not decline over time. When groups become too large, groups fission, often along kin lines. When groups split, females usually left with their own offspring, although in two cases adult females left with closely related kin that were not offspring. However, we did not find that Cape ground squirrels foraged closer to more closely related group members. Direct fitness benefits, as opposed to inclusive fitness and kinship, are more important for group splitting, but inclusive fitness and kinship might still be important in the sociality of Cape ground squirrels.

Introduction

Grouping is common in taxa ranging from birds to mammals and even insects (Clutton-Brock, 2002; Seppä *et al.*, 2008; McDonald et al., 2016). Group living provides several benefits, including thermal regulation, cooperative breeding, and decreased predation risk (Armitage, 1981; Vuren and Armitage, 1994). Some of the major benefits of antipredator behaviour are through the dilution effect, where the probability of an individual being preyed upon decreases with increasing group size, and the collective detection hypothesis, where overall group vigilance increases with larger groups because there are more animals to be vigilant for predators and warn groupmates (Armitage, 1981; Beauchamp, 2017, 2019; Clutton-Brock et al., 1999; Edwards and Waterman, 2011; Elgar, 1989). However, there are also costs to group living, such as increased competition for food and mates, disease transmission, increased risk of conspicuousness, inbreeding, and reproductive suppression (Dunbar, 2018). For gregarious females, increases in group size often result in decreased reproductive success (Clutton-Brock and Huchard, 2013; Dunbar, 2018; Lukas and Clutton-Brock, 2012). However, there are ways of mitigating the costs of group living, one of which is kin selection (Hatchwell, 2010).

Kin selection is a mechanism that may offset group costs through inclusive fitness (Smith, 1964). By helping close relatives or kin, an individual may propagate shared genes even at the expense of one's reproductive opportunities (Griffin and West, 2002; Hamilton, 1964; Smith, 2014). In this way, individuals could show preference to close kin (Aviles et al., 2004; Griffin and West, 2002; Smith, 2014), and individuals may rely on relatives more so than less related individuals to perform altruistic behaviour (Armitage, 1981).

Many mammals exhibit female philopatry, where females remain in the area they were born, resulting in kin groups or matrilines (Lukas et al., 2005). Female-biased kin relationships are important for the structuring and outcomes of social interactions in mammalian societies (Mattison et al., 2019; Briga et al., 2012). In groups where breeding is limited to a single individual or pair, matrilines will be made up of close female kin, and monogamy has been hypothesized to be a prerequisite to the evolution of cooperative breeding (Lukas and Clutton-Brock, 2012). However, in species with promiscuous mating systems, offspring are produced from multiple sires, and matrilines will contain a mosaic of kin relationships (Aviles et al., 2004; Dunbar, 2018; Lukas et al., 2005; Lukas and Clutton-Brock, 2012). In such promiscuous species, coefficients of relatedness are predicted to decrease with increasing group size and over time due to the influx of genes from many different mates (Aviles et al., 2004; Lukas et al., 2005; Lukas and Clutton-Brock, 2012).

In large groups of promiscuous breeders, the benefits of inclusive fitness could be overshadowed by the costs (Dunbar, 2018; Hatchwell, 2010; Lukas et al., 2005). However, individuals in a group could still gain inclusive fitness benefits if they bias their behaviour towards kin (Beauchamp, 2019; Dunbar, 2018; Hatchwell, 2010; Seppä et al., 2008). Such bias towards kin is only possible if individuals can discriminate their relatedness to other group members (Kramer and Russell, 2014). Some species with polyandrous mating systems form cooperative sub-groups based on kinship during activities like foraging because they can discriminate kinship on a finer scale (Best et al., 2014; Hatchwell, 2010; Jones, 2014; Kramer and Russell, 2014). Alternatively, large groups with low relatedness may split (Chepko-Sade and Sade, 1979). Leaving or dispersing is an important mechanism in dealing with the costs of group living, and how new groups form is fundamental to the genetics of a species (Cant et al., 2016). Clutton-Brock and Lukas (2012) describe three reasons for leaving the group, including avoidance of infanticide, inbreeding avoidance, and decreasing resource competition with group members and kin (Clutton-Brock and Lukas, 2012). Those individuals that leave can form a new group with lower competitive costs and continue benefiting from group living (Armitage et al., 2011; Dunbar, 2018; Seppä et al., 2008). If these groups split along kinship lines, they will also increase their inclusive fitness benefits (Walker and Hill, 2014).

Studies linking mating systems and the extent and form of kin discrimination are limited, especially in promiscuous mating systems (Cornwallis et al., 2009; Kramer and Russell, 2014; West et al., 2002). Detecting within-group kin biases may be difficult, depending on what is being measured, and few studies have examined kin biases in spatial associations during foraging in groups with varying relatedness (Hatchwell, 2010). Feeding may be risky when the behaviour occurs away from safe refuge or in high predation areas (Beauchamp, 2017, 2019). The benefit of collective detection relies on information conveyed by conspecifics (often via alarm calls) and the reliability of close kin is predicted to be higher than non-kin (Pollard and Blumstein, 2012). Thus, individuals may form sub-groupings of closer kin within a promiscuous social group during foraging (Charif et al., 2005; King, 1989; Maher, 2009; Podgórski et al., 2014; Stoen et al., 2005; Vuren and Armitage, 1994).

Cape ground squirrels (*Xerus inauris)* are a semi-fossorial, cooperative breeding species found in southern Africa that live in matrilineal kin clusters (Waterman, 1995; Waterman, 2002; Pettitt et al., 2008; Pettitt and Waterman, 2011). Female matrilines consist of 1-6 related adult females and their immature offspring and non-dispersed related adult males (Hillegass et al., 2008). The female matrilines form single family groups defined by the sharing of a sleeping burrow at night and a common foraging area in the day (Waterman, 1995; Waterman, 2002). Breeding is highly promiscuous, resulting in an array of kin relationships within a family group (Waterman, 1996; Waterman, 1998; Waterman and Archibald, 2019). In addition, female Cape ground squirrels can detect the degree of relatedness of individuals on a fine scale, although they do not seem to apply scent discrimination within their family groups (Waterman and Archibald, 2019). Most social interactions amongst a family group occur during the morning and evening and occur mainly near their sleeping burrow (Waterman, 1995; Unck *et al.*, 2009). However, during the day, family groups forage together and spend much of their time feeding away from the safety of their sleeping burrow, but remain in their family groups as protection from predation (Waterman, 1995; Unck *et al.*, 2009; Edwards and Waterman, 2011).

The objective of our study was to investigate patterns of relatedness and kin bias in the social Cape ground squirrel. We hypothesised that if promiscuous breeding affects group relatedness, then relatedness should be negatively related to group size, and relatedness within groups will decline over time (P1). If inclusive fitness is important in spatial proximity in this species, then (P2) larger groups will be more likely to experience group splitting compared to smaller groups, and (P3) the new groups formed will split along kin lines, resulting in higher relatedness in the new groups formed compared to the individuals left in the original group. In addition, if kinship is important during foraging (spatial distance), (P4) individuals will maintain closer proximity to their closest kin in the group, or (P5) in the case of kin conflict (or avoiding competition with kin), the closest kin will be found at greater distances apart than more distantly related group mates.

Methods

Biology of The Study Animal

Cape ground squirrel family groups live in burrow clusters defined as a group of openings closer to each other than any other burrows on the landscape (Herzig-Straschil, 1978). The burrow clusters are sometimes characterized by disturbance vegetation and a sunken-in, bowl-shaped appearance that is easy to distinguish from the surrounding landscape (Ewacha et al., 2016). A family groups’ home range may encompass more than one burrow cluster, but only a single-family group inhabits a burrow cluster at any one time (Waterman, 1996). During the day, squirrels spend most of their time away from the safety of the burrow cluster to forage for food (Waterman, 1995). Burrow clusters are used for sleeping and some social interaction only in the morning and late evening when the squirrels return from foraging (Waterman, 1995).

Unlike North American ground squirrels, Cape ground squirrels are not constrained by breeding seasons, and breeding occurs throughout the year (Waterman 2002). Females are philopatric, usually remaining in their natal group (Waterman 2002). Males often disperse at maturity (10 months) and join all-male bands that only interact with family groups during mating (Waterman, 1995; Manjerovic and Waterman, 2015). Not all males disperse at maturity, as some will delay dispersal (natal males) and remain in their family groups for up to 5 years, but inbreeding within family group members is rare (Pettitt and Waterman, 2011; Manjerovic and Waterman, 2015). Female Cape ground squirrels mate with an average of 4 males in an approximately 3hr estrus, and the mean operational sex ratio during estrus is high (11 males to 1 female) (Waterman, 1998; Manjerovic and Waterman, 2015). Females can become sexually mature as early as 7-9 months, but reproductive suppression can delay sexual maturity up to 14 months, and both the number of adult females (greater than three) and natal males in the group influence this suppression (Waterman, 1996; Waterman, 2002; Pettitt and Waterman, 2011). Females isolate from their family group in a separate burrow to give birth and will usually rejoin the family group when the young are weaned (Waterman 1995; Waterman 2002). No evidence of infanticide has been described (Waterman, pers. comm.). Group size may be constrained by resource competition, as per capita reproductive success decreases with increasing group size at a site in Namibia and larger groups have larger home ranges (Waterman, 2002).

Study Site and Trapping

We collected data during April-September 2017, the austral winter and dry season, on SA Lombard Nature Reserve (27º 36’ 6.48’’S, 25º 28’ 0.48’’ E) in central South Africa, where squirrels have been studied since 2002. In 2017, winter ambient temperatures ranged from -3.5ºC at night to 27.8ºC during the day, and the site received 552mm of rain during the rainy season.

We trapped squirrels using live traps (15x15x50cm, Tomahawk Live Trap Co., Tomahawk, WI, USA) baited with peanut butter and bird seed. We handled squirrels using cloth handling bags to minimize stress and contact (Koprowski 2002; Pettitt and Waterman 2011). Body mass was measured using a spring scale (Pesola, Baar, Switzerland). We painted a unique dye mark onto the dorsal area to identify individuals from a distance (Rodol D, Lowenstein and Sons Inc., New York, NY; Waterman 1995; Pettitt and Waterman 2011), and we implanted a passive integrated transponder (AVID Inc., Norco, CA) for permanent identification. We assessed reproductive condition by visual inspection of the nipples and genitalia of females (Waterman 1995). To approximate the age class of female squirrels, we examined their nipples, as they swell with the first estrus and remain swollen for the life of the squirrel (Waterman 1995). Subadults (immature animals) do not have swollen nipples or descended scrotum (Waterman 1995; Pettitt et al. 2008). A small piece of skin (<3mm) from the tail tip was removed during the first capture for genetic analysis to determine relatedness and maternity (Manjerovic and Waterman 2015).

Observations

In 2017, we observed family groups for 4 hours in the morning when they emerged, and from 4-6 hours in the afternoon until they immerged into their burrows in the evening. We used a random number generator in R 3.6.0 (R Core Team, 2019) to select which family group to observe each day. We observed squirrels using either an observation tower or a hide on top of a car, and observations took place 40 to 100m away from the focal group of squirrels. We used binoculars (10x50, Legacy, Bushnell Co. KS, USA) and spotting scopes (15-45x60mm, Spacemaster, Bushnell Co. KS, USA) to observe the behaviour and location of squirrels. We collected observation data using scan sampling every 10 min (Altmann, 1974), and recorded the location, identity, and behaviour (ethogram from Waterman, 1995) of each squirrel visible in the focal family group at the time of each scan using an Android application designed for this project.

Spatial Data Collection and Analysis

We recorded the location of squirrels using a Cartesian grid denoted by painted rocks in a north-south, east-west axis that were spaced 10 m apart (Waterman 1995). Additional rocks were added to the grid depending on squirrel movements, as deemed appropriate through observations. This grid system allowed us to determine an approximate coordinate of a squirrel to within one meter (Waterman, 1995). The center of the grid (0,0 coordinate) was recorded with a GPS unit (Garmin, GPSMAP78, Kansas US), to convert the positions of the squirrels into projected real-world coordinates in ArcMap 10.5 (ESRI 2017, CA: Environmental Systems Research Institute).

We also recorded the size and shape of burrow clusters used by family groups by recording GPS coordinates every 3 m around the perimeter of the burrow cluster. We used these points to digitize polygon vector data of every burrow cluster in use by the family groups in 2017. The environment is not saturated and there were empty burrow clusters that were not in use by any squirrels (Waterman, 1996; Manjerovic, 2010).

We used a projection of lo-25 in the program ArcMap10.5 and converted all squirrel locations into the coordinates of this projection. All area and distance calculations were performed on the projected coordinates. In our calculations of distance between individuals during foraging, we did not include behaviours while squirrels were at the burrow cluster. The time spent on the burrow cluster would bias our proximity calculations between squirrels towards 0 m, as squirrels emerge and immerge in the same burrow opening and stay within one meter of each other to socialize while on the burrow cluster, making it difficult to discern a kin bias through a spatial perspective (Herzig-straschil, 1978; Waterman 1995; Waterman 2002). In addition, the squirrels do not prioritize foraging while at the burrow cluster (Waterman, 1995).

Using the Garmin GPS, we collected information on the size and number of burrow clusters in use by squirrel groups to create burrow cluster boundaries. We found that the average size of the burrow clusters was highly variable (115.46 ± 22.2 m^2, $n = 28$), and the number of burrow clusters in use by a family group ranged from 1 to 4 (2.0 ± 0.31 burrow clusters). To examine the distance between squirrels during foraging, we used the data on burrow cluster size and number to exclude any squirrel locations that fell within burrow cluster boundaries by using the “clip” tool in ArcMap 10.5 (ESRI 2017, CA: Environmental Systems Research Institute). We determined the average distance between pairs of squirrels during foraging by calculating the Euclidean distance of each pair, within a family group, every 10 min after they had moved away from the burrow cluster area. We averaged the resulting distance measure per day for each pair of squirrels within a family group.

Genetics

We extracted DNA using an E.Z.N.A ® Tissue DNA Kit (Omega Bio-tek, Inc., Norcross, Ga, USA). We amplified the DNA with polymerase chain reactions and fluoresced 20 microsatellite loci specific to Cape ground squirrels (Shave and Waterman 2017; Abercrombie et al. 2009). We sent the amplified and marked DNA to be sequenced at the Center for Applied

Genomics (Hospital for Sick Children, Toronto, Canada), and we manually scored each allele using GeneMarker (v2.6.0; Hulce et al. 2011).

We used GENECAP (v1.4; Wilberg and Dreher, 2004), and our long-term database (2002-2017), to ensure that there were no duplicate individuals. We estimated pairwise relatedness between squirrel dyads with COANCESTRY (v1.0.1.8; Wang 2011) using the R Studio package *related* v1.0 (Pew et al., 2015). We used the triadic likelihood estimator, *trioml*, which was most highly correlated to our true data, to estimate pairwise relatedness using 100,000 bootstrap permutations (Shave and Waterman 2017). Coefficients of relatedness or relatedness values range from 0 – 1, where 0 is no alleles in common (not related), and 1 is all alleles in common (Shave and Waterman 2017). Finally, we used the program CERVUS v3.0.7 to use maximum likelihood estimation to assign parentage to the offspring (Kalinowski, Taper, and Marshall, 2007). We used 100,000 iterations using the allele frequency analysis generated in CERVUS. All adult females in the group were used as potential mothers, and all adult males in the area were included as possible fathers in the parentage assignment (Manjerovic and Waterman, 2015). We selected the option to run the analysis with both paternal and maternal sexes known, and our results did not deviate from Hardy-Weinberg equilibrium. We only accepted parentage assignments at 80% confidence or higher (Manjerovic and Waterman 2015).

Group Fission

We defined a matriline as a focal female squirrel and her direct female descendants in a family group. As squirrels on our study site have been followed since 2002 – apart from 2008-2010, when we did not have access to the study site – we were able to determine the age and follow matrilines of individuals within family groups across study years. We recorded all the individuals in each family group (including natal males) and the burrow cluster they occupied. We counted all group splitting events (fissions, when members of the group in one year were in a new family group the next year) and recorded both the individuals that left the group and the individuals who remained in the group after the split during all study years. We calculated an average coefficient of relatedness and the number of squirrels for three subgroups: (1) before the split, which includes the family group or “original group” before squirrels fissioned to form a new group in a different burrow cluster; (2) after the split, which includes the remaining squirrels that did not leave the original group; and (3) the new group that includes the newly separated squirrels living in a different burrow cluster.

Statistics

We calculated all statistics in R v3.3.1 (R core team 2016). We ran all generalized linear mixed models (GLMM) and linear mixed models (LMM) using the *lmer4* package (Kuznetsova and Christensen 2017). We further assessed these models using the *DHARMa* package (Hartig, 2016). Where there were no *P* values for GLMM, we used the *lmerTest* package to add *P* values (Kuznetsova, Brockhoff, and Christensen, 2017), or a post hoc analysis of variance (ANOVA) in the *car* package (Fox and Weisber, 2019).

To assess how promiscuous breeding affected group relatedness (P1), we ran two linear mixed models (LMM) fit by restricted maximum likelihood (REML;Harville, 1977) to examine how relatedness changed over time and with different group sizes. We divided the analysis into family group which included natal males and just matrilines, because males are the dispersing sex and eventually leave the family group and may not be influenced by kin selection to the same degree as the philopatric sex (Waterman, 1995). For both analyses concerned with relatedness, year, and group size, we used group identity as a random effect.

To compare the average coefficient of relatedness between the original, the individuals remaining of the original group after group splitting, and the new group (P3), we used a Kruskal-Wallis test, as the data violated the assumption of equal variance. We did not exclude natal males from this analysis, as squirrels may have formed a new group with males. We used a post hoc pairwise Wilcoxon rank-sum test for multiple comparisons. We conducted a similar analysis using group size data that did not violate the assumptions of an analysis of variance (ANOVA), and we used an ANOVA to compare original group size, the individuals remaining of the original group after splitting, and the new group. In this case, we used a post hoc Tukey's honest significant difference (HSD) test for multiple comparisons. To assess the importance of group size on the persistence of groups (whether they split or not) from one year until the next (P2), we analyzed group size and the likelihood of group splitting using a restricted maximum likelihood (REML) generalized linear mixed model (GLMM; Harville, 1977). We considered a family group stayed the same size from one year to the next year if the size had not increased or decreased by more than one animal. We considered groups to have increased if they added at least two individuals. We considered a group to decrease in size from one year to the next if they split (where individuals are found nearby), or decreased by at least two individuals, where these individuals were not found on the study site. Females move only a short distance if they leave their group, and we have never seen females disperse over a longer distance (Waterman 2002). Thus, if a female is no longer present in the area, we are confident she died (Waterman 2002). We are similarly confident that young males that disappear are either dead or dispersed. Our data were not normal, and we determined a Poisson distribution best fit our group size data. To analyse the persistence of groups (P2) we used the family group ID and year as random effects, and a post hoc pairwise Wilcoxon rank-sum test with a Bonferroni adjustment to further determine which means in our results were significantly different.

Finally, to assess how kinship influenced foraging distance (P4 and P5), we conducted two GLMMs with family group, including natal males and matrilines. Our data best fit a negative binomial distribution. We used two random effects: unique ID to identify squirrel pairs and family group ID to identify which squirrel pairs belonged to which family group. Using a GLMM also allowed assessment of competition, as our results would show a negative trend if more closely related squirrels would be found farther apart. All means reported are accompanied by standard errors unless otherwise specified (mean ± SE), and we used an alpha value of 0.05 to determine significance.

Results

The average number of females of all ages within a group was 3.53 ± 0.42 (range = 2 – 7; n = 15 groups) individuals. Group size, including males of all ages, was 6.07 ± 0.71 (range = 2 – 12; n = 15 groups) individuals. The mean pairwise relatedness among females within a group was 0.27 ± 0.03 (range = 0.0 – 0.74; n = 81 pairs of females in 15 family groups). If we included family group natal males, the mean pairwise relatedness dropped to 0.23 ± 0.01 (range = 0.0 – 0.74, n = 275 pairs of squirrels in 15 family groups). We followed 26 matrilines and their family groups consecutively for a minimum of 3 years from 2002 to 2017, and we found that total group relatedness (including males) decreased with increasing group size and decreased over time (LMM, R^2 = 0.28, Table 1, Figure 1 and 2). When we considered matrilines, we found that relatedness in matrilines decreased with increasing group size but did not change significantly over time (LMM, R^2 = 0.17, Table 1, Figure 1 and 2).

We recorded 13 splitting events (where squirrels left their original group and formed a new group), which account for 9.5% (n = 137) of groups monitored between 2002 and 2017. On average, 2.0 ± 0.31 (range 2 – 3 squirrels) squirrels would split off from a group, and all events (100%) had at least one older adult female who left the group with a younger individual. The average distance between two adjacent burrow clusters was 53.6 ± 2.8m and the average distance between the burrow cluster of the new group and their original group's burrow cluster was 127.5 ± 20.5m (range = 20.0 – 288.8). However, 30.77% of new groups moved into a burrow cluster immediately adjacent to their family group. In 84.6% (11/13) of splitting events, the squirrels that split were either mother-offspring pairs or single adult females that had isolated to give birth and did not return to their family group upon weaning their offspring. The relationships in the two other splitting events (15.38%) included one aunt-nephew and two half-sisters.

Within-group relatedness differed among the original group, the new group, and the individuals who remained of the original group after the split (Kruskal-Wallis test: $\chi^2 = 8.76$, $P = 0.013$, df = 2, Figure 3). The relatedness of the new group differed from the original group before the split (pairwise Wilcoxon rank-um, $P = 0.020$) and from the original group remaining after the split ($P = 0.036$). There was no difference between the average coefficient of relatedness of the original group before the split and the individuals remaining in the original group after the split ($P = 0.64$). We also found that there was a difference in group size among the original group, the new group, and the individuals remaining in the original group after the split (ANOVA, $F_{(2,36)} = 11.61$, $P < 0.001$, Figure 4). The new group was significantly smaller than the original group before the split (Tukey's HSD test, $P < 0.001$), and the individuals remaining in the original group ($P = 0.003$). We did not find a difference in group size between the original group before the split and the original group after the split ($P = 0.52$).

We tested our prediction that larger groups are more likely to split from one year to the next from 2002 to 2017 and categorized our data into three groups: groups that split, groups that did not split but reduced for unknown reasons, and groups that remained the same or increased from one year to the next. The average size of groups that split was 6.5 ± 0.6 (range = 4 – 12) individuals, while groups that decreased in size (but did not split) had an average of 6.26 ± 0.49 (range = 1 – 14) individuals. Groups that stayed the same or increased from one year to the next had an average of 4.66 ± 0.25 (range = 1 – 11) individuals. Groups that eventually split or decreased were significantly larger than groups that remained the same or increased (Poisson GLMM, estimate = 0.052, $\chi2$ = 20.3, df = 140, *P* < 0.001). Groups that split were larger than groups that remained the same or increased (pairwise Wilcoxon rank-um test, *P* = 0.015). There was no difference between groups that split and groups that reduced for unknown reasons (*P* = 1.00).

Within a group, the mean distance between female squirrel pairs, once they had left the burrow cluster to forage, was 21.2 ± 1.69 m (range from 1.00 – 64.44 m; n = 80 squirrel pairs). In comparison, the mean distance between squirrel pairs, including males, once they left the burrow cluster to forage was 19.8 ± 0.74 m (range = 1 – 101.5 m, n = 270 squirrel pairs). We did not find a relationship between relatedness and proximity of female foraging pairs, as they foraged away from the safety of the burrow clusters (negative binomial GLMM, estimate = 0.14, $\chi2$ = 1.43, df = 1162, *P* = 0.40). We conducted a similar analysis on family group that included natal males and still did not find that there was a relationship between relatedness and proximity, as pairs foraged away from the safety of burrow clusters (negative binomial GLMM, estimate = 1.44, $\chi2$ = 0.70, df = 346, *P* = 0.23).

Discussion

We investigated patterns of relatedness and kin bias in a facultative cooperative breeder with a promiscuous mating system. We found that promiscuous mating did impact average group relatedness and group size over time, but the change in relatedness over time occurred only when natal males were included and not when considering only matrilines. When splitting from a group, adult female squirrels usually left with their offspring and did not move far from their original group. Average relatedness was higher and group size was smaller in the new group compared to the original group. We did not find any support for kin-biased spatial proximity during foraging away from the burrow.

As expected for a promiscuous species, we found that average squirrel relatedness decreased with increasing group size as the more breeders within the group, the less likely pairwise relatedness will remain high (Aviles et al., 2004; Lukas et al., 2005). In particular, in female lion prides (*Panthera leo*), which consist of plural breeders living in family groups similar to Cape ground squirrels, average relatedness decreases with greater numbers of adult females in the group, indicating higher levels of promiscuity and lower levels of inbreeding than expected (Spong et al., 2002). White sifaka (*Propithecus verreauxi verreauxi*) that live in philopatric female kin groups of 2 - 14 animals and have male biased dispersal patterns, also demonstrate that as group size increases average relatedness decreases (Lawler et al., 2003). Females in this species, have a dominance hierarchy which stratifies the breeding opportunities among females (Lawler et al., 2003). This is unlike that of Cape ground squirrels who do not have a female dominance hierarchy for mating opportunities, yet relatedness responds the same to increasing groups size between these two species. As the number of female sifakas increases in a group, there is an increased influx of genetic material as females seek out unrelated male partners to avoid inbreeding, and as a result, individuals are less related to each other (Lawler et al., 2003). However, the opposite relationship occurs in European rabbits *(Oryctolagus cuniculus),* where average relatedness increases with increasing group size. This increase in relatedness was due to a lack of males immigrating into the group and a lack of males emigrating out of the group, which led to increased inbreeding over the course of the study (Surridge et al., 1999). Our results were more reminiscent of the results found in lions then that of the European rabbit, as inbreeding is very rare in Cape ground squirrels (1 of 137 offspring; Manjerovic and Waterman, 2015) and, young females in Cape ground squirrels avoid inbreeding with natal males (Waterman, 2002; Jackson et al., 2007; Pettitt and Waterman, 2011).

After controlling for group size, average group relatedness (including males) declined over time in Cape ground squirrels, but when we excluded males, and only examined matrilines, we did not find that average female group relatedness changed over time. For males, over time they may become less related to their group members because of female promiscuous breeding and related females leaving to form new groups. Additionally, as male Cape ground squirrels do not breed within their own family group, they will never have their own highly related offspring in their group. Subsequent individuals born into the group are therefore less related to any natal males, resulting in an overall decreased group relatedness. A similar pattern is seen in Superb fairy-wrens *(Malurus splendens)* that have natal male helpers. Relatedness between the male helpers and offspring decreased over time, as the probability of death or dispersal of close maternal relatives (mother) of the male helpers increased over time (Dunn et al., 1995). Natal male Cape ground squirrels rarely breed with females in their own family groups, instead they seek reproductive opportunities in nearby groups (Manjerovic and Waterman, 2015).

For females, relatedness may not decline over time because multiple females are having their own offspring in the group. Often, the relatedness of philopatric females in a group is higher than males, even in promiscuous species (Matocq and Lacey, 2004; Van Horn *et al.*, 2004; Lukas *et al.*, 2005). Specifically, in savannah baboons *(Papio ursinus)* females directly recruit daughters into their family groups (Van Horn et al., 2007). Similar recruitment is seen in yellow-bellied marmots (*Marmota flaviventris*) where breeding females recruit their own offspring into their family groups, which usually consist of a single adult female, her yearling offspring and young (matriline) and one male (Armitage and Schwartz, 2000; Armitage et al., 2011). In these examples, maternal relatedness is high within the group due to the recruitment of daughters, but these analyses did not follow relatedness over time. In spotted hyenas *(Crocuta crocuta),* the average relatedness of matrilines is higher within a matriline than among matrilines (Van Horn *et al.*, 2004). However, over time, female promiscuity in spotted hyena decreases the average relatedness of matrilines as immigrant males join the group and breed within the matrilines diluting matrilineal relatedness (Van Horn *et al.*, 2004). In Cape ground squirrels, the relatedness of groups is influenced by both promiscuous breeding and the fission of family groups.

Indeed, the formation of new groups by leaving with direct descendants may result in conservation of matrilineal relatedness despite plural breeding. Groups splitting along kinship lines, resulting in increased relatedness in new groups, is found in big brown bats *(Eptesicus fuscus),* lions, and black-tailed prairie dogs (*Cynomys ludovicianus*) (Manno et al., 2007; Metheny et al., 2008; Packer et al.,1991). New groups in Cape ground squirrels were usually established by an adult female and her offspring, and these groups had higher average relatedness than the original group. Group size was also smaller in the new group compared to the original group. Black-tailed prairie dog females also live "coteries" or family groups consisting of plural breeding female kin and 1 – 2 non-breeding yearlings (Manno et al., 2007). Like Cape ground squirrels, young male black-tail prairie dogs disperse at maturity (Hoogland, 1995). Black-tailed prairie dogs also exhibit a pattern where, when groups split, average relatedness increases slightly, although not significantly, compared to the original group (Manno et al., 2007). However, five of the new groups were formed by adult females who left the coterie to give birth and potentially start a new matriline (Manno et al., 2007). This pattern is consistent with the pattern seen in our study, as females often isolated to give birth and never returned to the group. Black-tailed prairie dogs that leave their group may gain direct fitness benefits by splitting with their daughters when there were few close kin in their original territory (Manno et al., 2007). There are many documented cases of adult females gaining direct fitness benefits by helping their offspring to survive or to reduce resource or reproductive competition (Arnaud et al., 2012; Chepko-Sade and Sade, 1979; Price and Boutin, 1993; Stoen et al., 2005). For example, in Columbian ground squirrels (*Urocitellus columbianus*) adult females tolerate spatial overlap with their daughters, as competition for nesting sites and breeding territories are intense within a colony (Arnaud et al. 2012). In this way, adult females gain direct fitness benefits by helping their daughters become established through the sharing of critical breeding territory (Arnaud et al. 2012). In red squirrels (*Tamiasciurus hudsonicus*), a territory is vital to the survival of female red squirrels, and adult females have been known to bequeath territory to their daughters (Price and Boutin, 1993). More specifically, females that gave birth later in the year were more likely to bequeath territory to their late-born daughters, as these offspring are at a disadvantage to growing and finding territory compared to earlier-born offspring (Price and Boutin, 1993). This bequeathal is a direct fitness advantage for adult female red squirrels with late-born daughters (Price and Boutin, 1993). In yellow-bellied marmots, adult females delay dispersal until enough closely related maternal kin are present within the group, at which point the matriline leaves (Armitage and Schwartz, 2000). In this species, a certain number of maternal kin are required before fission can occur to ensure the resulting new group has enough individuals to protect against predation pressure (Armitage and Schwartz, 2000). The formation of new groups with their offspring by female Cape ground squirrels is consistent with maximizing direct fitness and may also give a fitness advantage to their subsequent and future generations, especially when groups become large.

There are several possible reasons for group splitting when groups become too large, especially for females (Clutton-Brock and Lukas, 2012). First, females may choose to leave the group to avoid infanticide, such as in white-headed leaf monkeys (*Trachypithecus leucocephalus*) and in Ursine colobus monkeys (*Colobus vellerosu*) (Teichroeb et al., 2009; Zhao et al., 2011). Both studies indicate that females left the group to protect their offspring when a new male had taken control of their group (Zhao et al., 2011). In black-tailed prairie dogs, infanticide is a significant reason why females may leave their family group (Manno et al., 2007). In Cape ground squirrels, females isolate from their family group to give birth but usually return to the group once young emerge (Waterman, 1995). They also do not have a dominant male monopolizing and defending breeding females in the group and there is no evidence for infanticide in this species (Waterman, unpubl. data).

Another reason females may choose to leave their family group is to decrease competition within the family group, particularly with kin (Clutton-Brock and Lukas, 2012; Manno et al., 2007). Competition for resources and breeding opportunities among kin can negate any benefits of kin selection (Dunbar, 2018; West et al., 2002) and competition for breeding opportunities can lead to reproductive suppression (Clutton-Brock and Lukas, 2012). Such reproductive suppression can be a result of inbreeding avoidance, because inbreeding may lead to decreased heterozygosity and result in less viable offspring (Clutton-Brock and Lukas, 2012; Nichols, 2017). To avoid inbreeding, females may disperse to increase mating opportunities with non-kin (Nichols, 2017). In Yunnan snub-nosed monkeys (*Rhinopithecus bieti*), females disperse from their family group seeking unrelated males to breed with (Wan-Cai et al., 2020). In Cape ground squirrels, more than two related adult males in a family group delay sexual maturity in subadult females, most likely as a form of inbreeding avoidance through self-restraint (Jackson et al., 2007; Pettitt and Waterman, 2011).

Cape ground squirrel immature females are also reproductively suppressed by the presence of more than three breeding adult females (Waterman, 2002; Pettitt and Waterman, 2011). The presence of breeding females influenced the age of maturity of young subadult females who would normally mature at 8 months to up to 12 to 14 months (Waterman, 2002; Pettitt and Waterman, 2011). Such suppression could have lifetime consequences (Armitage et al., 2011). In yellow-bellied marmots, mothers may delay the first reproduction of daughters beyond sexual maturity, costing daughters in lifetime fitness (Armitage et al., 2011). Thus, avoiding reproductive suppression could be one reason for a mother to form a new group with their daughter, as the daughters would mature and breed more rapidly. However, the presence of female kin can delay female dispersal due to inclusive fitness benefits, or even direct fitness (Clutton-Brock and Lukas, 2012). Yellow-bellied marmot daughters who stay with their mothers have two possible strategies when group size becomes large: disperse to avoid increased competition (and reproductive suppression) or remain philopatric (Armitage et al., 2011). Chances of survival in these young marmots decreases with dispersal. Daughters who had a mother present in the matriline, who interacted amicably with their mother, and whose space use overlapped with their mother, were more likely to remain philopatric, despite being reproductively suppressed (Armitage et al., 2011). In this way, mothers maximize their direct fitness by retaining daughters in their natal group, and daughters also benefit from the protection and resources available by remaining philopatric, increasing their survival (Armitage et al., 2011). It is unclear what cues yellow-bellied marmot females use to determine when to disperse, but kinship is a significant factor in keeping daughters philopatric (Armitage et al., 2011). In Cape ground squirrels, larger groups are the ones that split. This pattern is consistent with results from a study in Namibia, where groups that split were larger, but it was the younger female squirrels that split from the group due to reproductive suppression (Waterman, 2002). Relatedness between these young females was unavailable at the time. In our study, while most new groups were formed by mothers and their offspring, in two events older females formed new groups with highly related but indirect descendants, suggesting the possibility of kin selection.

If kinship is important during foraging (spatial distance), we predicted individuals would maintain closer proximity to their closest kin in the group, or in the case of kin conflict (or avoiding competition with kin), the closest kin will be found at greater distances apart than more distantly related group mates. We did not find that that either was the case, as there was no relationship between foraging proximity and relatedness. This lack of pattern might be due to two reasons: prior familiarity and/or group augmentation due to predation pressure. Prior familiarity is often used as a relatively accurate proxy for relatedness in many species, as some species cannot detect relatedness on a fine degree (Mateo, 2003; Nichols, 2017). In Seychelles warbler (*Acrocephalus sechellensis*), a cooperatively breeding bird, individuals determine who they are related to using the prior familiarity of birds that fed them when they were young (Komdeur et al., 2004). It is a relatively good proxy for determining which birds are related, because it is usually the mother and/or a related subordinate female that usually feeds the young (Komdeur et al., 2004). Upon reaching adulthood subordinate females may remain philopatric and gain inclusive fitness benefits by helping the birds that fed them (Komdeur et al., 2004). A recent study examining kin discrimination in female Cape ground squirrels demonstrated that they do not use kin detection through odour within their family group, and only demonstrated the ability to detect kin of varying degrees with who they were not familiar with (Waterman and Archibald, 2019). Similarly, Belding's ground squirrels *(Spermophilus beldingi)* can detect relatedness on a fine scale, but the ability to do so does not always lead to nepotistic behaviours (Mateo, 2002, 2003). Since matriline relatedness in Cape ground squirrels is stable over time, squirrels may just use prior familiarity as a proxy for relatedness within their group.

The act of foraging for Cape ground squirrel comes with increased risk of predation as they move away from their burrow cluster (Edwards and Waterman, 2011). Predation is a prominent pressure for Cape ground squirrels, and mutual cooperation to avoid predators might be greater than any benefits received from kin selection (Clutton-Brock *et al.*, 1999; Clutton-Brock, 2002; Cheney *et al.*, 2010; Smith, 2014; Candiotti *et al.*, 2015). Larger groups sizes have reduced vigilance, supporting both the collective detection and dilution hypotheses as important benefits of group living in Cape ground squirrels (Edwards and Waterman, 2011; Unck et al., 2009). Vigilance has in the past been thought of as kin-selected behaviour in ground squirrels, where individuals perform costly vigilant behaviour and alarm calling for close kin (Dunford, 1977; Sherman, 1985). But alternative explanations, such as group augmentation have recently been suggested to explain the benefits of grouping and vigilance during foraging (Kingma et al., 2014). The group augmentation hypothesis states that any behaviour, where all members receive direct benefit from living in larger groups, will persist (Kingma et al., 2014). The resulting behaviour may supersede the need for kin selection (Smith, 2014). For example, meerkats (*Suricata suricatta)* take turns acting as sentinels (Clutton-Brock et al., 1999). The more meerkats available, the more members there are to share sentinel responsibility, and there is no need for the joining group members to be kin as all additional members, regardless of kinship, can exhibit sentinel behaviour (Clutton-Brock et al., 1999). Several studies have demonstrated how kin are not always selected to offset group costs (Clutton-Brock *et al.*, 1999; Charif *et al.*, 2005; Candiotti et al., 2015; Quirici et al. 2013). Campbell's monkeys (*Circopithecus campbelli*) showed no preferential kin bias in spatial proximity (Candiotti et al., 2015). Degus (*Octodon degus*) use sentinel behaviour when foraging with partners, but kinship is a poor predictor of coordinated vigilance efforts between foraging pairs (Quirici et al., 2013). Like Cape ground squirrels, kin directed behaviour in degus may be overridden by the direct benefits that group vigilance has for every individual (Quirici et al., 2013). Overall, prior familiarity and the benefits of group vigilance are both possible reasons for a lack of kin-biases in the spatial behaviour of Cape ground squirrels when they forage away from the safety of the burrow cluster.

In conclusion, promiscuous breeding does impact group relatedness, but not always. In the case of Cape ground squirrel matrilines, other factors can keep relatedness from decreasing over time, such as group fission, and maximizing direct fitness by forming new groups with offspring. Our findings contrast with studies that suggest that social vertebrates that give rise to complex behaviour like cooperative breeding are monogamous, due to the maintenance of high group relatedness (Lukas and Clutton-Brock, 2012, 2018). Inclusive fitness does not appear to be as important to Cape ground squirrels as much as direct fitness benefits such as splitting along direct descent lines, although we also saw the possibility of fission along indirect descent lines. In addition, kinship may also not be important during foraging, as Cape ground squirrels do not preferentially forage with the closest kin in the group, most likely due to the stability of relatedness over time and predation pressure. Future studies might consider understanding kin bias through a social interaction perspective rather than a spatial one.

References

Abercrombie, L. G., Anderson, C. M., Baldwin, B. G., Bang, I. C., Beldade, R., Bernardi, G., … Yuan, J. S. (2009). Permanent genetic resources added to molecular ecology resources database 1 january 2009-30 april 2009. *Molecular Ecology Resources*, *9*(5), 1375–1379. https://doi.org/10.1111/j.1755-0998.2009.02746.x

Altmann, J. (1974). Observational study of behavior: sampling methods. *Behaviour*, *49*(3–4), 227–266. https://doi.org/10.1163/156853974X00534

Armitage, K. B. (1981). Sociality as a life-history tactic of ground squirrels. *Oecologia*, *48*, 36–49.

Armitage, K. B., & Schwartz, O. A. (2000). Social enhancement of fitness in yellow-bellied marmots. *Proceedings of the National Academy of Sciences*, *97*(22), 12149–12152. https://doi.org/10.1073/pnas.200196097

Armitage, K. B., Vuren, D. H. Van, Ozgul, A., and Oli, M. K. (2011). Proximate causes of natal dispersal in female Yellow-bellied marmots, Marmota flaviventris. *Ecology, 92*(1), 218–227.

Arnaud, C. M., Dobson, F. S., and Murie, J. O. (2012). Philopatry and within-colony movements in Columbian ground squirrels. *Molecular Ecology*, *21*(3), 493–504. https://doi.org/10.1111/j.1365-294X.2011.05219.x

Avilés, L., Fletcher, J. A., & Cutter, A. D. (2004). The Kin Composition of Social Groups: Trading Group Size for Degree of Altruism. *The American Naturalist*, *164*(2), 132–144. https://doi.org/10.1086/422263

Beauchamp, G. (2017). The spatial distribution of foragers and food patches can influence antipredator vigilance. *Behavioral Ecology*, *28*(1), 304–311. https://doi.org/10.1093/beheco/arw160

Beauchamp, G. (2019). On how risk and group size interact to influence vigilance. *Biological Reviews*, *94*(6), 1918–1934. https://doi.org/10.1111/brv.12540

Best, E. C., Dwyer, R. G., Seddon, J. M., & Goldizen, A. W. (2014). Associations are more strongly correlated with space use than kinship in female eastern grey kangaroos. *Animal Behaviour*, *89*, 1–10. https://doi.org/10.1016/j.anbehav.2013.12.011

Briga, M., Pen, I., & Wright, J. (2012). Care for kin: within-group relatedness and allomaternal care are positively correlated and conserved throughout the mammalian phylogeny. *Biology Letters*, *8*(4), 533–536. https://doi.org/10.1098/rsbl.2012.0159

Candiotti, A., Coye, C., Ouattara, K., Petit, E. J., Vallet, D., Zuberbühler, K., & Lemasson, A. (2015). Female bonds and kinship in forest guenons. *International Journal of Primatology*, *36*(2), 332–352. https://doi.org/10.1007/s10764-015-9829-1

Cant, M. A., Nichols, H. J., Thompson, F. J., and Vitikainen, E. (2016). Banded mongooses: demography, life history, and social behaviour. In W. Koenig and J. Dickinson (Eds.), *Cooperative breeding in vertebrates: studies of ecology, evolution, and behaviour* (pp. 318–337).

Charif, R. A., Ramey, R. R., Langbauer, W. R., Payne, K. B., Martin, R. B., and Brown, L. M. (2005). Spatial relationships and matrilineal kinship in African savanna elephant (Loxodonta africana) clans. *Behavioural Ecology and Sociobiology*, *57*(4), 327–338. https://doi.org/10.1007/s00265-004-0867-5

Cheney, D. L., Moscovice, L. R., Heesen, M., Mundry, R., & Seyfarth, R. M. (2010). Contingent cooperation between wild female baboons. *Proceedings of the National Academy of Sciences*, *107*(21), 9562–9566. https://doi.org/10.1073/pnas.1001862107

Chepko-Sade, B. . D., and Sade, D. S. (1979). Patterns of group splitting within matrilineal kinship groups : a study of social group structure in Macaca mulatta (Cercopithecidae : Primates). *Behavioural Ecology and Sociobiology*, *5*(1), 67–86. Retrieved from https://www.jstor.org/stable/4599218

Clutton-Brock, T. H., and Lukas, D. (2012). The evolution of social philopatry and dispersal in female mammals. *Molecular Ecology*, *21*(3), 472–492. https://doi.org/10.1111/j.1365-294X.2011.05232.x

Clutton-Brock, T., and Huchard, E. (2013). Social competition and its consequences in female mammals. *Journal of Zoology*, *289*(3), 151–171. https://doi.org/10.1111/jzo.12023

Clutton-Brock, T. (2002). Breeding together: kin selection and mutualism in cooperative vertebrates. *Science*, *296*(5565), 69–72. https://doi.org/10.1126/science.296.5565.69

Clutton-Brock, T. H., Riain, M. J. O., Brotherton, P. N. M., Gaynor, D., Kansky, R., Griffin, A. S., and Manser, M. (1999). Selfish sentinels in cooperative mammals. *Science*, *284(5420)*, 1640–1645.

Cornwallis, C. K., West, S. A., and Griffin, A. S. (2009). Routes to indirect fitness in cooperatively breeding vertebrates: kin discrimination and limited dispersal. *Journal of Evolutionary Biology*, *22*(12), 2445–2457. https://doi.org/10.1111/j.1420-9101.2009.01853.x

Dunbar, R. I. M. (2018). Social structure as a strategy to mitigate the costs of group living : a comparison of gelada and guereza monkeys. *Animal Behaviour*, *136*, 53–64. https://doi.org/10.1016/j.anbehav.2017.12.005

Dunford, C. (1977). Kin selection for ground squirrel alarm calls. *The American Naturalist*, *111*(980), 782–785. https://doi.org/10.1086/283206

Dunn, P. O., Cockburn, A., & Mulder, R. A. (1995). Fairy-wren helpers often care for young to which they are unrelated. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *259*(1356), 339–343. https://doi.org/10.1098/rspb.1995.0050

Edwards, S., & Waterman, J. M. (2011). Vigilance and grouping in the southern African ground squirrel (Xerus inauris). *African Journal of Ecology*, *49*(3), 286–291. https://doi.org/10.1111/j.1365-2028.2011.01262.x

Elgar, M. A. (1989). Predator vigilance and group size in mammals and birds: a critical review of the empirical evidence. *Biological Reviews*, *64*(1), 13–33. https://doi.org/10.1111/j.1469-185X.1989.tb00636.x

Ewacha, M. V. A., Kaapehi, C., Waterman, J. M., & Roth, J. D. (2016). Cape ground squirrels as ecosystem engineers: modifying habitat for plants, small mammals and beetles in Namib Desert grasslands. *African Journal of Ecology*, *54*(1), 68–75. https://doi.org/10.1111/aje.12266

Fox, J., and Weisber, S. (2019). *An R Companion to Applied Regression*. Retrieved from https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Griffin, A. S., & West, S. A. (2002). Kin selection: fact and fiction. *Trends in Ecology & Evolution*, *17*(1), 15–21. https://doi.org/10.1016/S0169-5347(01)02355-2

Hamilton, W. D. (1964). The genetical evolution of social behaviour. II. *Journal of Theoretical Biology*, *7*(1), 17–52. https://doi.org/10.1016/0022-5193(64)90039-6

Hartig, F. (2016). *DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models*. Retrieved from https://cran.r-project.org/package=DHARMa

Harville, D. A. (1977). Maximum likelihood approaches to variance component estimation and to related problems. *Journal of the American Statistical Association*, *72*(358), 320–338. https://doi.org/10.1080/01621459.1977.10480998

Hatchwell, B. J. (2010). Cryptic Kin selection: kin structure in vertebrate populations and opportunities for kin-directed cooperation. *Ethology*, *116*(3), 203–216. https://doi.org/10.1111/j.1439-0310.2009.01732.x

Herzig-straschil, B. B. (1978). On the biology of Xerus inauiris (Zimmermann, 1780) (Rodentia, Sciuridae). *Z. Säugetierkunde*, *43*, 262–278.

Hillegass, M. A., Waterman, J. M., & Roth, J. D. (2008). The influence of sex and sociality on parasite loads in an African ground squirrel. *Behavioral Ecology*, *19*(5), 1006–1011. https://doi.org/10.1093/beheco/arn070

Hoogland, J. L. (1995). *The black-tailed prairie dog: social life of a burrowing mammal*. Chicago, Illinois: University of Chicago Press.

Jackson, T. P., Waterman, J. M., & Bennett, N. C. (2007). Pituitary luteinizing hormone responses to single doses of exogenous GnRH in female social Cape ground squirrels exhibiting low reproductive skew. *Journal of Zoology*, *273*(1), 8–13. https://doi.org/10.1111/j.1469-7998.2007.00333.x

Jones, C. B. (2014). *The Evolution of Mammalian Sociality in an Ecological Perspective*. Ashville, North Carolina: Springer International Publishing, 2014.

Kalinowski, S. T., Taper, M. L., and Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, *16*(5), 1099–1106. https://doi.org/10.1111/j.1365-294X.2007.03089.x

King, W. J. (1989). Spacing of female kin in Columbian ground squirrels (Spermophilus columbianus). *Canadian Journal of Zoology*, *67*(1), 91–95. https://doi.org/10.1139/z89-014

Kingma, S. A., Santema, P., Taborsky, M., & Komdeur, J. (2014). Group augmentation and the evolution of cooperation. *Trends in Ecology & Evolution*, *29*(8), 476–484. https://doi.org/10.1016/j.tree.2014.05.013

Komdeur, J., Richardson, D. S., & Burke, T. (2004). Experimental evidence that kin discrimination in the Seychelles warbler is based on association and not on genetic relatedness. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *271*(1542), 963–969. https://doi.org/10.1098/rspb.2003.2665

Koprowski, J. L. (2002). Handling tree squirrels with a safe and efficient restraint. *Wildlife Society Bulletin (1973-2006)*, *30*(1), 101–103.

Kramer, K. L., & Russell, A. F. (2014). Kin-selected cooperation without lifetime monogamy: human insights and animal implications. *Trends in Ecology & Evolution*, *29*(11), 600–606. https://doi.org/10.1016/j.tree.2014.09.001

Kuznetsova, A., Brockhoff, P. B., and Christensen, R. H. B. (2017). lmerTest package: tests in linear mixed effects models. *Journal of Statistical Software*, *82*(13), 1–26. https://doi.org/10.18637/jss.v082.i13

Laporte, F., and Mary-Huard, T. (2017). *Relatedness: Maximum Likelihood Estimation of Relatedness using EM Algorithm*. Retrieved from https://cran.r-project.org/package=Relatedness

Lawler, R. R., Richard, A. F., & Riley, M. A. (2003). Genetic population structure of the white sifaka (Propithecus verreauxi verreauxi) at Beza Mahafaly Special Reserve, southwest Madagascar (1992–2001). *Molecular Ecology*, *12*(9), 2307–2317. https://doi.org/10.1046/j.1365-294X.2003.01909.x

Lukas, D., Reynolds, V., Boesch, C., & Vigilant, L. (2005). To what extent does living in a group mean living with kin? *Molecular Ecology*, *14*(7), 2181–2196. https://doi.org/10.1111/j.1365-294X.2005.02560.x

Lukas, D., & Clutton-Brock, T. (2012). Cooperative breeding and monogamy in mammalian societies. *Proceedings of the Royal Society B: Biological Sciences, 279*(1736), 2151–2156. https://doi.org/10.1098/rspb.2011.2468

Lukas, D., & Clutton-Brock, T. (2018). Social complexity and kinship in animal societies. *Ecology Letters*, *21*(8), 1129–1134. https://doi.org/10.1111/ele.13079

Maher, C. R. (2009). Genetic relatedness and space use in a behaviorally flexible species of marmot, the woodchuck (Marmota monax). *Behavioral Ecology and Sociobiology*, *63*(6), 857–868. https://doi.org/10.1007/s00265-009-0726-5

Manjerovic, M. B. (2010). *The Influence Of Sexual Selection On Behavioural And Physiological Mechanisms Underlying Reproductive Success In Male Cape Ground Squirrels (Xerus inauris)*. University of Central Florida.

Manjerovic, M. B., & Waterman, J. M. (2015). "Failure to launch": is there a reproductive cost to males living at home? *Journal of Mammalogy*, *96*(1), 144–150. https://doi.org/10.1093/jmammal/gyu015

Manno, T. G., Dobson, F. S., Hoogland, J. L., & Foltz, D. W. (2007). Social group fission and gene dynamics among Black-tailed prairie dogs (Cynomys ludovicianus). *Journal of Mammalogy*, *88*(2), 448–456. https://doi.org/10.1644/06-MAMM-A-099R1.1

Mateo, J. M. (2002). Kin-recognition abilities and nepotism as a function of sociality. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *269*(1492), 721–727. https://doi.org/10.1098/rspb.2001.1947

Mateo, J. M. (2003). Kin recognition in ground squirrels and other rodents. *Journal of Mammalogy*, *84*(4), 1163–1181. https://doi.org/10.1644/BLe-011

Matocq, M. D. (2004). Philopatry, kin clusters, and genetic relatedness in a population of woodrats (Neotoma macrotis). *Behavioral Ecology*, *15*(4), 647–653. https://doi.org/10.1093/beheco/arh056

Mattison, S. M., Shenk, M. K., Thompson, M. E., Borgerhoff Mulder, M., & Fortunato, L. (2019). The evolution of female-biased kinship in humans and other mammals. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*(1780), 20190007. https://doi.org/10.1098/rstb.2019.0007

McDonald, P. G., Rollins, L. A., and Godfrey, S. (2016). The relative importance of spatial proximity, kin selection and potential 'greenbeard' signals on provisioning behaviour among helpers in a cooperative bird. *Behavioural Ecology and Sociobiology*, *70*, 133–143. https://doi.org/10.1007/s00265-015-2032-8

Metheny, J. D., Kalcounis-Rueppell, M. C., Bondo, K. J., & Brigham, R. M. (2008). A genetic analysis of group movement in an isolated population of tree-roosting bats. *Proceedings of* *the Royal Society B: Biological Sciences*, *275*(1648), 2265–2272. https://doi.org/10.1098/rspb.2008.0532

Nichols, H. J. (2017). The causes and consequences of inbreeding avoidance and tolerance in cooperatively breeding vertebrates. *Journal of Zoology*, *303*(1), 1–14. https://doi.org/10.1111/jzo.12466

Packer, C., Gilbert, D. A., Pusey, A. E., & O'Brieni, S. J. (1991). A molecular genetic analysis of kinship and cooperation in African lions. *Nature*, *351*(6327), 562–565. https://doi.org/10.1038/351562a0

Pettitt, B. A., & Waterman, J. M. (2011). Reproductive delay in the female Cape ground squirrel (Xerus inauris). *Journal of Mammalogy*, *92*(2), 378–386. https://doi.org/10.1644/10-MAMM-A-168.1

Pettitt, B. A., Waterman, J. M., & Wheaton, C. J. (2008). Assessing the effects of resource availability and parity on reproduction in female Cape ground squirrels: resources do not matter. *Journal of Zoology*, *276*(3), 291–298. https://doi.org/10.1111/j.1469-7998.2008.00491.x

Pew J., Muir P.H., Wang J., Frasier T.R. (2015). related: an R package for analyzing pairwise relatedness from codominant molecular markers. *Molecular Ecology Resources* 15: 557-561.

Podgórski, T., Lusseau, D., Scandura, M., Sönnichsen, L., & Jędrzejewska, B. (2014). Long-lasting, kin-directed female interactions in a spatially structured wild boar social network. *PLoS ONE*, *9*(6), e99875. https://doi.org/10.1371/journal.pone.0099875

Pollard, K. A., & Blumstein, D. T. (2012). Evolving communicative complexity: insights from rodents and beyond. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367*(1597), 1869–1878. https://doi.org/10.1098/rstb.2011.0221

Price, K., & Boutin, S. (1993). Territorial bequeathal by red squirrel mothers. *Behavioral Ecology*, *4*(2), 144–150. https://doi.org/10.1093/beheco/4.2.144

Quirici, V., Faugeron, S., Hayes, L. D., & Ebensperger, L. A. (2011). The influence of group size on natal dispersal in the communally rearing and semifossorial rodent, Octodon degus. *Behavioral Ecology and Sociobiology*, *65*(4), 787–798. https://doi.org/10.1007/s00265-010-1082-1

R Core Team. (2019). *R: A Language and Environment for Statistical Computing*. Retrieved from https://www.r-project.org/

Seppä, P., Fernández-Escudero, I., Gyllenstrand, N., & Pamilo, P. (2008). Colony fission affects kinship in a social insect. *Behavioral Ecology and Sociobiology*, *62*(4), 589–597. https://doi.org/10.1007/s00265-007-0483-2

Shave, J. R., & Waterman, J. M. (2017). The effects of familiarity and reproductive status on olfactory discrimination by female Cape ground squirrels (Xerus inauris). *Behavioral Ecology and Sociobiology*, *71*(12), 184. https://doi.org/10.1007/s00265-017-2410-5

Sherman, P. W. (1985). Alarm calls of Belding's ground squirrels to aerial predators: nepotism or self-preservation? *Behavioral Ecology and Sociobiology*, *17*(4), 313–323. https://doi.org/10.1007/BF00293209

Smith, J. E. (2014). Hamilton's legacy: kinship, cooperation and social tolerance in mammalian groups. *Animal Behaviour*, *92*, 291–304. https://doi.org/10.1016/j.anbehav.2014.02.029

Smith, J. M. (1964). Group selection and kin selection. *Nature*, *201*(4924), 1145–1147. https://doi.org/10.1038/2011145a0

Spong, G., Stone, J., Creel, S., & Björklund, M. (2002). Genetic structure of lions (Panthera leo L.) in the Selous Game Reserve: implications for the evolution of sociality. *Journal of Evolutionary Biology*, *15*(6), 945–953. https://doi.org/10.1046/j.1420-9101.2002.00473.x

Støen, O-G., Bellemain, E., Sæbø, S., & Swenson, J. E. (2005). Kin-related spatial structure in brown bears Ursus arctos. *Behavioral Ecology and Sociobiology*, *59*(2), 191–197. https://doi.org/10.1007/s00265-005-0024-9

Surridge, A. K., Ibrahim, K. M., Bell, D. J., Webb, N. J., Rico, C., & Hewitt, G. M. (1999). Fine-scale genetic structuring in a natural population of European wild rabbits (Oryctolagus cuniculus). *Molecular Ecology*, *8(2)*, 299–307.

Teichroeb, J., Wikberg, E., & Sicotte, P. (2009). Female dispersal patterns in six groups of ursine colobus (Colobus vellerosus): infanticide avoidance is important. *Behaviour*, *146*(4–5), 551–582. https://doi.org/10.1163/156853909X426363

Unck, C. E., Waterman, J. M., Verburgt, L., & Bateman, P. W. (2009). Quantity versus quality: how does level of predation threat affect Cape ground squirrel vigilance? *Animal Behaviour*, *78*(3), 625–632. https://doi.org/10.1016/j.anbehav.2009.05.028

Van Horn, R. C., Engh, A. L., Scribner, K. T., Funk, S. M., & Holekamp, K. E. (2004). Behavioural structuring of relatedness in the spotted hyena (Crocuta crocuta) suggests direct fitness benefits of clan-level cooperation. *Molecular Ecology*, *13*(2), 449–458. https://doi.org/10.1046/j.1365-294X.2003.02071.x

Van Horn, R. C., Buchan, J. C., Altmann, J., & Alberts, S. C. (2007). Divided destinies: group choice by female savannah baboons during social group fission. *Behavioral Ecology and Sociobiology*, *61*(12), 1823–1837. https://doi.org/10.1007/s00265-007-0415-1

Van Vuren, D., & Armitage, K. B. (1994). reproductive success of colonial and noncolonial female Yellow-bellied marmots (Marmota flaviventris). *Journal of Mammalogy*, *75*(4), 950–955. https://doi.org/10.2307/1382476

Walker, R. S., & Hill, K. R. (2014). Causes, consequences, and kin bias of human group fissions causes, consequences, and kin bias of human group fissions. *Human Nature*, *25*(4), 465–475. https://doi.org/10.1007/s12110-014-9209-0

Wang, J. (2011). Coancestry: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Molecular Ecology Resources*, *11*(1), 141–145. https://doi.org/10.1111/j.1755-0998.2010.02885.x

Waterman, J. M. (1995). The Social organization of the Cape ground squirrel (Xerus inauris; Rodentia: Sciuridae). *Ethology, 101*(2), 130–147.

Waterman, J. M. (1996). Reproductive biology of a tropical, non-hibernating ground squirrel. *Journal of Mammalogy*, *77*(1), 134–146. https://doi.org/10.2307/1382715

Waterman, J. M. (1998). Mating tactics of male Cape ground squirrels, Xerus inauris: consequences of year-round breeding. *Animal Behaviour, 56*(2), 459–466. https://doi.org/10.1006/anbe.1998.0780

Waterman, J. M. (2002). Delayed maturity, group fission and the limits of group size in female Cape ground squirrels (Sciuridae: Xerus inauris). *Journal of Zoology*, *256*(1), 113–120. https://doi.org/10.1017/S0952836902000146

Waterman, J. M., & Archibald, A. J. (2019). Both familiarity and kinship influence odour discrimination by females in a highly social African ground squirrel. *Animal Behaviour*, *148*, 145–151. https://doi.org/10.1016/j.anbehav.2018.12.007

West, S. A., Pen, I., & Griffin, A. S. (2002). Cooperation and competition between relatives. *Science*, *296*(5565), 72–75. https://doi.org/10.1126/science.1065507

Wilberg, M. J., and Dreher, B. . (2004). *GENECAP : a program for analysis of multilocus genotype data for non-invasive sampling and capture-recapture population estimation*. 783–785. https://doi.org/10.1111/j.1471-8286.2004.00797.x

Wan-Cai, X., Sheng-Nan, J., Bao-Ping, R., Xin-Ming, H., Tai, Z., Ali, K., … Da-Yong, L. (2020). Proximate causes of dispersal for female Yunnan snub-nosed monkeys. *Zoological Research*, *41*(1), 78–83. https://doi.org/10.24272/j.issn.2095-8137.2020.008

Zhao, Q., Borries, C., & Pan, W. (2011). Male takeover, infanticide, and female countertactics in white-headed leaf monkeys (Trachypithecus leucocephalus). *Behavioral Ecology and Sociobiology*, *65*(8), 1535–1547. https://doi.org/10.1007/s00265-011-1163-9

Figures and Tables

Table 2.1: Results of the linear mixed models examining if average group relatedness was affected by time and group size in the Cape ground squirrel. There are two linear mixed models in which natal males were included (LMM, R^2 marginal = 0.28), and excluded (LMM, R^2 marginal = 0.17).

Family group (Including natal males)					
Fixed effect	**Estimate**	**Std. Error**	***df***	***t-value***	***P***
Year	-0.014	0.0055	107.96	-2.68	0.0086*
Group Size	-0.025	0.0039	105.71	-6.47	<0.001*
Matrilines					
Fixed effect	**Estimate**	**Std. Error**	**df**	**t-value**	**P**
Year	0.011	0.0078	96.41	1.40	0.16
Group Size	-0.030	0.0072	96.98	-4.21	<0.001*

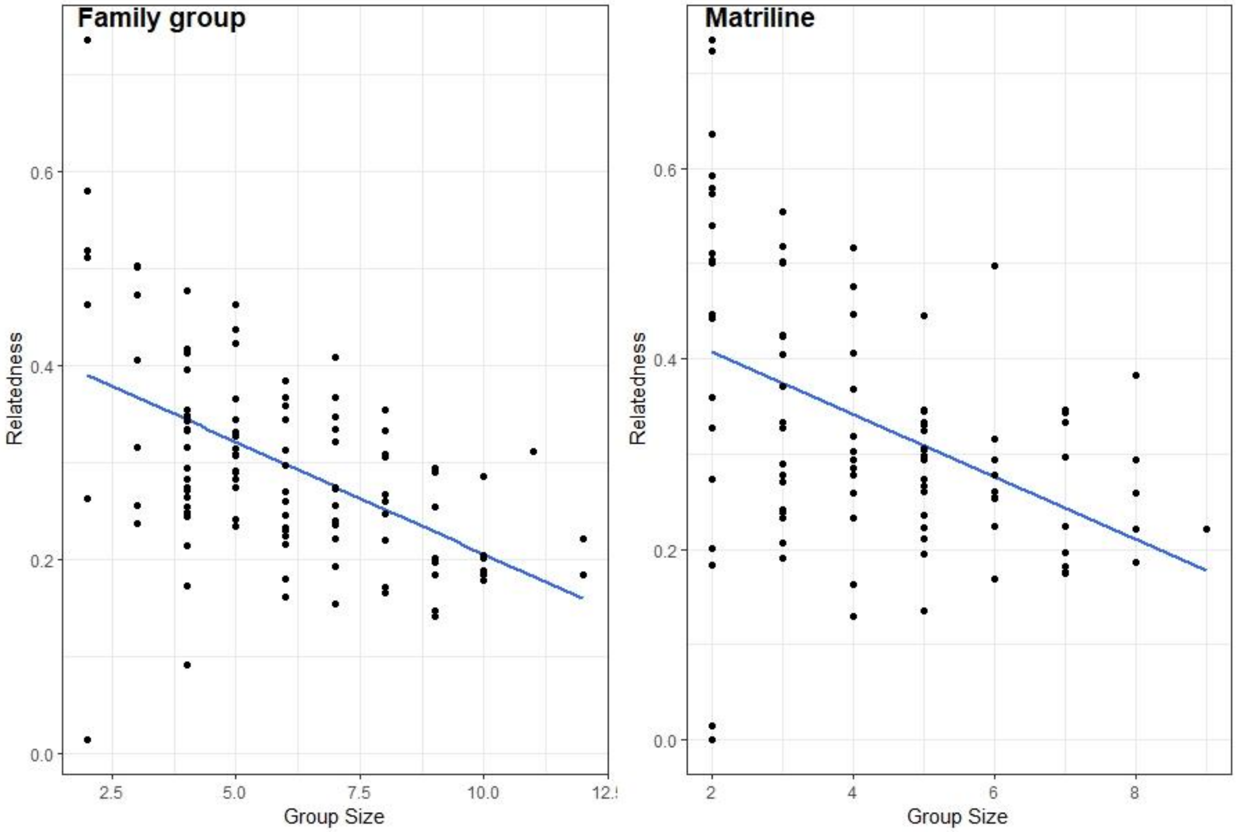

Figure 2.3: The negative relationship between average pairwise group relatedness and group size of Cape ground squirrel family groups including and excluding males (Males included LMM, R^2 marginal = 0.28, Table 1; Males excluded LMM, R^2 marginal = 0.17, Table 2).

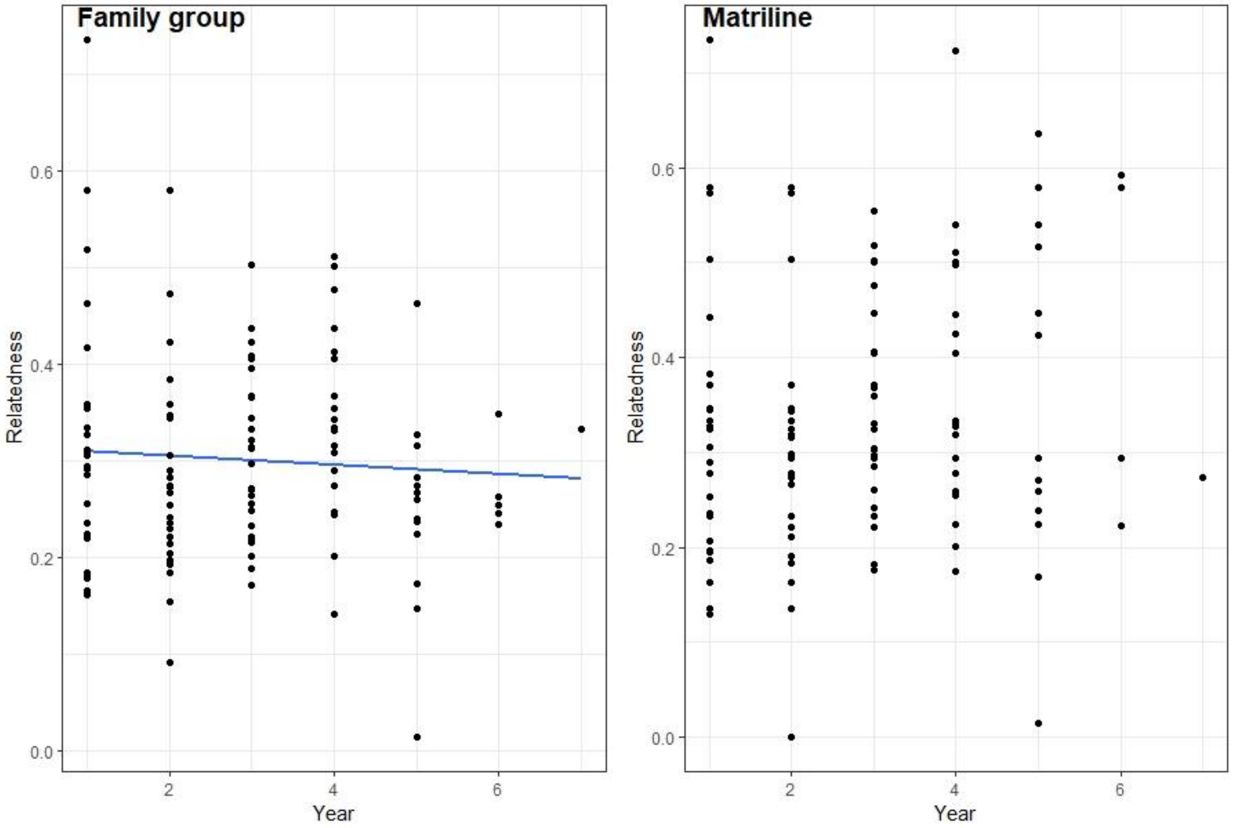

Figure 4.2: Average pairwise group relatedness over time in Cape ground squirrels. Results were only significant when males were included, and results were not significant when males were excluded (Males included LMM, R^2 marginal = 0.28, Table 1; Males excluded LMM, R^2 marginal = 0.17, Table 2).

Chapter 3: Lack of kin selection through space use among family groups of a cooperative breeding ground squirrel in southern Africa

Abstract

Group living has costs and benefits that can change as groups get larger. Several mechanisms offset group costs, such as kin selection, where individuals in a group favour those they are more closely related to because they may share more genes, termed inclusive fitness theory. Alternatively, groups may split once they get too large as the costs of group living start to outweigh the benefits. The isolation by distance hypothesis predicts that relatedness among individuals in a population decreases with increasing distance across the landscape. Therefore, social mammals that live in adjacent groups may still receive kin benefits from neighbouring groups. Cape ground squirrels (*Xerus inauris*) are a social species of ground squirrel that live in southern Africa in family groups made up of matrilines. However, they are non-aggressive and non-territorial even with neighbouring groups, unlike most other ground squirrel species. Our objective was to test the relatedness patterns of Cape ground squirrels across the landscape and determine if kin bias contributed to home range overlap, or if home range overlap contributed to female reproductive success and longevity. Our study took place on S.A. Lombard Nature Reserve, Southern Africa in 2017. We followed 15 family groups and collected position data through scan sampling observations. We found that Cape ground squirrel family groups followed an isolation by distance pattern, but squirrels did not show a kin bias in their home range overlap both within and among family groups. We also did not find that home range overlap contributed to reproductive success or longevity in female Cape ground squirrels. Overall, the size and distribution of home ranges of squirrel family groups might be tied to resource availability. Our study was conducted in a resource-rich year, so further research on home range overlap with differing resource availability is warranted.

Introduction

Group living can be the result of selective forces or random chance. Advantages to living in a group include predation protection (collective detection and the dilution effect), thermal regulation, cooperative breeding, and resource acquisition (Armitage, 1981; Wrona and Dixon, 1991; Majolo et al., 2008; Powers and Lehmann, 2016). However, there are also costs to group living, such as increased disease transmission, greater conspicuousness, competition for resources and reproductive suppression (Armitage 1981; Waterman 2002).

The fitness costs of group living can be decreased through kin selection, particularly in cooperative breeders (Armitage, 1981; Griffin and West, 2002; Mateo, 2002). Kin selection offsets group living costs by way of inclusive fitness where an individual will favour a close relative for altruistic behaviour because kin share similar genes. By helping relatives, individuals can indirectly pass on genetic information into the next generation (Hamilton, 1964). Across vertebrate taxa, those that help kin may experience direct benefits such as increased survival but may also experience indirect benefits such as increased survival and reproductive success of the kin they helped (Griffin and West, 2003; Hamilton, 1964). In many cooperatively breeding species, such as baboons (*Papio cynocephalus*), meerkats (*Suricata suricatta*), birds (*Aegithalos causatus*), and fish (*Neolamprologus pulcher*), increased care from relatives enhances infant survival and increases longevity (Silk et al., 2003; Russell *et al.*, 2007; Silk *et al.*, 2010; Zöttl et al., 2013; Green and Hatchwell, 2018).

In social mammals, fitness benefits often decline with increasing group size (Dunbar, 2018). Large groups may experience infanticide, inbreeding, and competition with group members and kin over resources (Clutton-Brock and Lukas, 2012). Many species cope with the costs of large group sizes by fissioning or splitting from original groups, for example in gelada baboons (*Theropithecus gelada*) fertility declines with increasing group size, and larger groups of gelada baboons will eventually split (Dunbar, 2018). After splitting from a group, the members of the new group may benefit by remaining close to their relatives left behind in the original group (Armitage, 2009; Dunbar, 2018; Stoen et al., 2005; Verdolin and Slobodchikoff, 2002). The isolation by distance hypothesis states that the greater the distance between groups, the less genetically similar they will be (Wright, 1943). We expect more closely related groups to be found closer together simply because dispersal distances are limited geographically (Wright, 1943). Nevertheless, kin that reside near each other might still benefit from kin bias, even if kin are spaced (Broquet et al.,2006). In Columbian ground squirrels (*Urocitellus columbianus*), females may not disperse far from their natal area, and tolerance of relatives may aid the new individual in reproduction (King, 1989). Indeed, females that have kin around them with both spatial and temporal overlap have better reproductive success then those that do not (Viblanc et al., 2010). In addition, individuals that remain close to related members may still gain other benefits such as food sharing and defence against predators (Armitage, 2009; Arnaud et al., 2012; Biondo *et al.*, 2014).

Space use can often be quantified using measures like home range, which can be a measure of the spatial resources that an organism needs to survive (Armitage, 2009). Sharing spatial resources or tolerance of others using spatial resources can have a kin-bias (Lambin and Krebs 1993; Ishibashi et al. 1997; Maher 2009; Johannesen et al. 2000; Stoen et al. 2005). Such kin preferences may determine the proximity in which kin groups settle near each other or how individuals within a group share space (Podgórski et al., 2014; Stoen et al., 2005). Territories in red squirrels (*Tamiasciurus hudsonic*) are usually highly food-based and are fiercely defended, but older females may bequeath or share their territories with their daughters (Price and Boutin, 1993). Indeed, relatedness may be a factor in determining how far individuals move away from their natal areas (Broquet et al., 2006). Detecting kin biases within and between family groups can be a challenge if direct interactions are rare but it can be approached through the analysis of their spatial dynamics. The analysis of spatial resources like home range in conjunction with fitness measures could indicate a fitness benefit due to kin selection (Lukas and Clutton-Brock, 2018). For example, yellow-bellied marmots (*Marmota flaviventris*) live in groups made up of female kin and the mothers in these groups exhibit social tolerance of daughters in their home range (Armitage et al.,2011). Younger yellow-bellied marmot females may experience reproductive suppression as a group cost and may choose to leave the group, but relatedness and the resulting benefits play a large role in the dispersal decisions of these young females (Armitage et al., 2011).

In many mammals, male-biased dispersal and female philopatry result in the formation of female matrilines, (Armitage, 1986; Dobson, 2013). Females who leave their natal group to avoid the costs of larger groups may stay relatively close to their natal area possibly to continue benefiting from kin selection (Stoen et al. 2005; Maher 2009). The Cape ground squirrel (*Xerus inauris)* is a social member of the family Sciuridae that lives in female matriline kin groups of 1-6 females and their immature offspring (Hillegass et al., 2008). Immature females in the group are sometimes constrained by reproductive suppression in the form of delayed maturity, which is influenced by both the number of adult females and related adult males in the group (Waterman 2002; Pettitt et al. 2011).

The splitting of matrilines in Cape ground squirrels has been attributed to reproductive suppression and resource competition (Waterman, 2002; Pettitt et al., 2008). Females usually move into an area near to their original group (Chapter 2). Young males that reach maturity (10 months) may disperse or they may remain in the family group (hereafter called natal males) and venture out during the day to find female mates from adjacent groups. Eventually, all males disperse from their family groups and join all-male roving bands (hereafter called band males; Manjerovic and Waterman, 2015). Males living in all-male bands share a home range and jointly search the area for receptive females (Waterman, 1997). The home range of a male band encompasses an average number of 30 female family groups (Manjerovic and Waterman, 2015). Consequently, relatedness among different family groups may be influenced through paternal relatedness, as one male can mate with many different females across a landscape (Manjerovic and Waterman, 2015). Due to the roaming nature of males and multiple mating by females, relatedness values among and within groups are highly variable (Waterman and Archibald, 2019). Cape ground squirrels are capable of discerning relatedness on a fine-scale but only do so with members outside of their family group (Waterman and Archibald, 2019). However, we do not know if Cape ground squirrels show intergroup kin bias in their use of space, or if the nearness of relatives in neighbouring groups affects the reproductive success and age of adult females. As the main benefit of grouping in this species appears to be enhanced predator avoidance via the dilution effect and collective detection (Waterman 1995, 1997), spatial proximity to kin from neighbouring groups could be important for survival. Kin occurring spatially close together can still direct amicable behaviour toward neighbouring kin in other family groups that could lead to potential fitness benefits later on, especially in prey species (Deng et al. 2019). Due to the rarity of intergroup behavioural interactions (Waterman 1995), an analysis of spatial proximity might be a better approach to discern if squirrels show a spatial preference towards kin among groups, and if these preferences influence squirrel reproductive success and survival.

The main objective of our study was to assess if kin influences the use of space among family groups. We hypothesized that if kin selection is an important selective force in Cape ground squirrels, then female groups that are related will benefit from proximity and have greater reproductive success and survival than compared to groups without close kin nearby groups. Specifically, we tested four predictions:

1) Closely related groups will have a shorter intergroup distance and follow an isolation by distance pattern.
2) Relatedness within a family group will be higher than among family groups.
3) The more closely related individuals are within and among groups, the greater the home range overlap within and between groups.
4) Individuals that share the most home range overlap among groups, will have greater longevity, and have a greater number of offspring.

Methods

Study Site

We collected data at S.A. Lombard Nature Reserve (27º 36’ 6.48’’S, 25º 28’ 0.48’’ E) from April-September 2017, during the austral dry season of central South Africa (Pettitt and Waterman, 2011). Temperatures ranged from -3.5ºC to 27.8ºC in the 2017 winter season. The S.A. Lombard Nature reserve receives an average of 502 mm of rain (1952-2004, Pettitt and Waterman, 2011) and during the rainy season in 2017, the site received 552 mm of rain. The 3659 ha study site is an open grassland flood plain, that consist of patches of tall perennial grass (*Cymbopogon plurinodis*), and shorter-wispier grass (*Themeda triandra*), or heavily-grazed areas, with a few scattered bushes (Herzig-straschil, 1978).

Biology of the Study Animal

Cape ground squirrels are semi-fossorial diurnal rodents that are active year-round (they do not hibernate; Waterman 1995). Squirrel groups live in burrow clusters, which are three or more burrow openings that are closer together to each other than to any other clusters of burrows on the landscape (Herzig-straschil, 1978). Family groups may include one or more burrow clusters in their home range, but only one family group uses a burrow cluster at a time (Waterman 1995). Family groups are identified by the individuals that emerge/immerge from a single burrow opening in the morning and evening respectfully (i.e., the individuals that sleep together; Waterman 1995). At any one time, there are empty burrow clusters throughout the flood plain, which suggests the habitat is not saturated (Waterman 1995). Cape ground squirrels eat grass seeds, roots and shoots in the top 5cm layer of soil (Herzig-straschil, 1978).

A family group in Cape ground squirrels consists of female matrilines of up to 5 adult females, two to three subadults of either sex, as well as 0-4 natal males (Waterman, 2002; Hillegass et al., 2008; Waterman and Archibald, 2019). Unlike many other social mammals, Cape ground squirrel family groups do not appear to have a dominance hierarchy, and aggression is rare (Waterman, 1995, 1996). Females are philopatric, breed asynchronously throughout the year, and multiply mate with up to 10 males in a single estrus (average of 4 males; Waterman, 1998). Males roam the veldt searching for females in scramble competition (Waterman, 1998), and the average operational sex ratio on the day of estrus is 11 males to 1 female (Manjerovic and Waterman, 2015). Multiple females breed within a group (plural breeders) and females isolate away from their family group to give birth and return only to their group when their young are weaned (Waterman, 1995). Their litter size is small (1-2 offspring), but 70% of litters of two are multiply sired (Manjerovic and Waterman, 2015). When groups get large, they split, with maternal kin moving to a nearby burrow cluster (Chapter 2).

Trapping and marking

We trapped animals using live traps (15cm x 15cm x 50cm; Tomahawk Live Trap Co., Tomahawk, WI, USA) baited with peanut butter and bird seed (Waterman 1995). We handled squirrels in cone bags, which allowed measurements to be taken with minimal stress (Koprowski, 2002). We used spring scales to measure body mass (Pesola, Switzerland), and each squirrel received a PIT tag (Passive Integrated Transponder tag, AVID Inc., Norco, CA) for permanent identification. To identify individuals from a distance, we painted a unique mark onto the back of individuals that was visible from a distance using black hair dye (Rodol D, Lowenstein and Sons Inc., New York, NY; Waterman 1995). We took 2-3mm of skin from the tail tip for all squirrels at the time of their first capture for genetic analysis (Manjerovic and Waterman, 2015).

We estimated the age of squirrels in years by using our long-term database from 2011-2017. Adult female squirrels are characterized by elongated nipples that remain swollen after first estrus (Waterman 2002). Subadults are immature animals that are not reproductive. Gestation and lactation are approximately 3 months combined, and females have their first estrus at approximately 10 months (Waterman, 1996; Pettitt and Waterman, 2011). If a squirrel at first capture was caught as an adult, we excluded them from the analysis.

Observations

In 2017 we observed 15 family groups. Observations started at the emergence of the family group at approximately 800 hrs until 1200 hrs, and again from 1300 hr - 1400 hrs until they immerged at night at approximately 1730 – 1800 hrs. We used a random number generator in the program R 3.6.0 (R Core Team, 2019) to determine which family groups were observed on each day. We used high vantage points (observation tower or a hide on top of a car), approximately 40-100m away from the focal family group for observation with 10 x 50 binoculars (Legacy, Bushnell Co. KS, USA) and 15-45x60mmm spotting scopes (Spacemaster, Bushnell Co. KS, USA). We collected data on the location (see Spatial Analysis) of all visible animals every 10 minutes using scan sampling (Altmann, 1974).

Spatial Analysis

We determined the location of individual squirrels by creating a Cartesian grid made up of painted rocks arranged in a North-South, East-West pattern across burrow clusters. The rocks were spaced every 10 meters and colour coded. We recorded a GPS coordinate at the center of the grid using a handheld Garmin 78GPS unit (Garmin, GPSMAP78, Kansas US). We were able to approximate a coordinate location of squirrels within one meter during observation by converting coordinates into the appropriate projection. We used a projection of lo-25 using the hartebeethoek94 datum in ArcMap 10.5 (ESRI 2017, CA: Environmental Systems Research Institute; National Geo-spatial Information, Dpt. Rural Development and Land Reform of South Africa). All spatial variables were calculated using the ArcMap 10.5 coordinate system (ESRI 2017, CA: Environmental Systems Research Institute).

We used the location data of squirrels to calculate all home ranges, and distances between home ranges. The centroids of home ranges of all 15 observed family groups were used to calculate the distance between all possible combinations of the 15 family groups resulting in 105 different distances among all family groups. Home ranges were estimated using the 95% Kernel Density Estimation method (Silverman, 1986) and were estimated using adehabitat (Calenge, 2006) in R 3.3.1 (R Core Team 2016). Any squirrel with fewer than 20 locations was excluded from the analysis as 20 locations would be too few points for an acceptable home range estimation (Mitchell, 2007). We combined the location data of all squirrels within a family group to estimate group home range. We used the reference bandwidth in the Kernel Density

Estimations as the spatial resolution was high (within 1m), and the data were in no danger of over-smoothing (Chu et al., 2015). We decided against using the plug-in bandwidth as it was overly conservative and the resulting home ranges from the plug-in bandwidth were patchy and tightly drawn around data points (Walter et al., 2011). Home ranges calculated in R 3.3.1 (R Core Team 2016), were imported into ArcMap to calculate home range overlap using the "tabulate intersection" tool in ArcMap 10.5 (ESRI 2017, CA: Environmental Systems Research Institute). Not all family groups had the potential to overlap. Therefore, we only included family groups that were within the diameter of an average home range (diameter = 118.44m, see results for average home range size) for our home range overlap analysis (prediction 2 and 3). Using the centroids for each of the home ranges, we used the "Buffer Analysis" tool in ArcMap10.5 (ESRI 2017, CA: Environmental Systems Research Institute), and selected all the family groups that were within the buffer zones of each other. To calculate squirrel density, we summed the area of all home ranges, and divided by the number of squirrels that contributed to the home ranges

Microsatellite DNA

DNA was extracted from tissue samples using E.Z.N.A ® Tissue DNA Kit (Omega Bio-Tek, Norcross, GA) then amplified the DNA using polymerase chain reactions (PCR) and fluoresced 20 microsatellite loci specific to Cape ground squirrels (Shave and Waterman 2017; Abercrombie et al. 2009) in the genetics lab at the Assiniboine Park Zoo. The Center for Applied Genomics (Hospital for Sick Children, Toronto, Canada) sequenced the amplified DNA, and we scored the genotypes using Genemarker (v.2.6.0).

We estimated pairwise relatedness between pairs of squirrels with the R package *related* v 1.0 (Pew *et al.*, 2015) based on the program Coancestry v 1.0.1.8 (Wang, 2011). As the triadic likelihood (*trioml)* estimator had the highest correlation with simulated data (Wang, 2011), we used this estimator and 10,000 bootstrap permutations to estimate the coefficients of relatedness (Shave and Waterman 2017). Coefficients of relatedness range from 0 to 1 where 0 indicates no alleles were shared between two pairs of squirrels to 1 where all alleles were shared between a pair of squirrels (Shave and Waterman 2017).

We used CERVUS v.3.0.7 (Marshall et al., 1998) to assign parentage to the offspring and tallied how many subadults were born to each female squirrel observed in 2017. CERVUS uses maximum likelihood estimation to determine parentage (Edwards, 1974). It compares simulated data to evaluate the confidence of parentage assignment over a specified number of iterations; we used 100,000 (Shave and Waterman, 2017). We tested individuals where both maternal and paternal parentage were unknown but selected the option where sexes of the parents were known. Individuals were typed at 10 or more loci, with an 84% success rate at 80% confidence level with parent pair (Manjerovic and Waterman, 2015). We used all adult females in family groups that were observed in 2017 as potential mothers, and all adult males trapped throughout the study site in 2017 were used as potential fathers (Manjerovic and Waterman, 2015). We used CERVUS to confirm that our population was in Hardy-Weinberg equilibrium.

Statistical Analysis

All statistical analyses were conducted in R 3.3.1 (R Core team 2016). We used a combination of generalized linear mixed models fit by maximum likelihood and linear mixed model fit by restricted maximum likelihood (REML) to account for collinearity between data points (Harville, 1977). For prediction 1 (isolation by distance) we used a linear mixed model, and because a suitable distribution could not be found, the data were log-transformed. We used the *lmer4* package (Kuznetsova and Christensen 2017), and a unique joint ID that included the colony of both squirrels within a pair as the random effect. To test if there was a difference in average relatedness within family groups versus adjacent family groups (prediction 2), we used a Wilcoxon signed-rank test as our data violated the equality of variance, and normality assumptions. For prediction 3, concerning home range overlap among and within family groups we used the *glmmADMB* package (Fournier et al., 2012; Skaug et al., 2016) to run beta distributed Generalized linear mixed models (GLMM) using unique joint ID that included the tag number between both squirrels as a random effect. Finally, when assessing home range overlap impacts on fitness among family groups (prediction 4), we used an LMM and a GLMM's fitted to the Poisson distribution and we used a unique ID as a random effect for both models. To assess the fit of the mixed models, we used the *DHARMa* package to generate diagnostic residual plots (Hartig, 2016). The *glmmADMB* was not supported with the *DHARMa* package and we extracted and plotted the residuals for model diagnostics. We used an ɒ= 0.05 as a threshold for significance.

Results

Female group size, including all ages, ranged from 2 – 7 (mean = 3.53 ± 0.42 squirrels) per family group. The average home range size of female squirrels was 0.68 ± 0.11 ha (n = 44; range = 0.018 – 2.46 ha). Average home range size of an entire family group that included all individuals of all ages was 1.10 ± 0.19 ha, (range = 0.14 – 2.59 ha, n = 15). Squirrel density, including natal males, was 4.8 squirrels/ha. There was no linear relationship between family group home range size and total family group size (linear regression, R^2= 0.0073, F_{13} = 0.10, P = 0.76, n = 15 family groups). There was also no linear relationship between individual female squirrel home range size, and family group size (LMM, estimate = -0.06 ± 0.11, t = -0.57, P = 0.57, n = 44).

The average distance between the centroid of family groups, where there was potential for home range overlap, was 153.3 ± 14.5 m. Among family groups, the average coefficients of relatedness among groups decreased with increasing distance (linear mixed model, estimate = -1.18e-04 ± 4.028e-06, t = -2.92, P = 0.0043, n = 105; Figure 3.1). Among family groups, the average coefficient of relatedness was lower than within groups when we analyzed females only (Figure 3.2) (Wilcoxon signed-rank test, W = 2841, $P < 0.0001$) or when we included males (Wilcoxon signed-rank test, W = 6351, $P < 0.0001$). Within a family group, the home range of females overlapped by 63.2 ± 2.00 % (range = 1.4 – 100 %). We did not find a relationship between home range overlap and relatedness of females within a family group (beta GLMM, estimate = 0.001 ± 0.34, z = 0.02, P = 0.98, n = 248). Among family groups, females who were not in the same family group, but had the potential to have overlapping home ranges shared on average 1.43 ± 0.46 % (range 0 – 12.94%) of their home ranges with squirrels in other family groups. We did not find a relationship between home range overlap among female squirrels in different family groups and how related they were (beta GLMM, estimate = 0.31 ± 0.51, z-value = 0.6, P = 0.55, n = 46, Figure 3.3).

The average age of all female squirrels in 15 family groups was 1.99 ± 0.24 yrs (range 0.26 - 5.63 yrs). The average age of adult female squirrels was 3.95 ± 0.20 yrs (range 1.84-5.63 yrs). We did not find a relationship between home range overlap and squirrel age (indicative of survival; Poisson GLMM, estimate = -0.0042 ± 0.0047, *z-value* = -0.93, P = 0.36, n = 77). The per capita number of offspring per females in 2017 was 1.38 ± 0.12 (range = 1 – 3). We did not find a relationship between home range overlap of family groups and the per capita number of offspring (Poisson GLMM, estimate = - 0.00052 ± 0.072, *z-value* = -0.072, *P* = 0.94, n= 64).

Discussion

We examined whether kin selection influences spatial patterns among female squirrel groups and if those spatial patterns influence reproductive success or longevity. We found that there was isolation by distance in this population of Cape ground squirrels and family groups were less related the greater the distance between them. Additionally, average relatedness was higher within family groups than among family groups. We did not find a relationship between home range overlap and the pairwise relatedness of squirrels both within and adjacent family groups. We also did not find that there was a relationship between home range overlap by way of increased per capita offspring or the age of female Cape ground squirrels.

Our first prediction, that more related squirrel groups will be geographically closer together, was supported. The pattern that geographically near family groups are more closely related is an isolation by distance pattern (Wright, 1943; Francisco et al., 2009). Sociality in female Cape ground squirrels has been characterized by female kin groups (Waterman 1995) and females also settle near other kin when they leave their natal groups (Waterman 2002; Chapter 2). Average relatedness within family groups was much higher than between family groups. However, the average distance between neighbouring family groups appeared farther than kin distances observed in other squirrel species, albeit species with different social structures from Cape ground squirrels. Michener (1979) found that during the active season of adult Richardson's ground squirrels (*Urocitellus richardsonii*), the nearest neighbour was between 20 – 25 m away, and in Columbian ground squirrels (*Spermophilus columbianus)* the distance between active centers of kin was 10.2 – 16.2 m, and younger females would nest more than 20 m away from other older females (King, 1989). However, behaviourally Richardson's and Columbian ground squirrels exist in large colonies, and females have small territories within the colony (King, 1989; Michener, 1979). Black-tailed prairie dogs *(Cynomys ludovicianus*) live in kin groups called coteries (family groups), which are similar to the family groups of Cape ground squirrels (Manno et al., 2007). When new coteries form, the home range is divided, and the new coteries live within 20 m of each other. Since subadult female Cape ground squirrels are reproductively suppressed in larger groups, individuals who split from a group might be predicted to settle further away from their original group (Waterman, 2002). Also, resources might be less abundant relative to that in black-tailed prairie dogs, and Cape ground squirrels must travel farther for resources (Waterman, 2002), as the distance between centroids of the Cape ground squirrel family group and their nearest neighbour were higher than black-tailed prairie dog coteries that split. Black-tailed prairie dog home ranges tend not to change in size over time despite coterie size changing suggesting high resource availability (Manno et al., 2007). Like Cape ground squirrels, black-tailed prairie dogs are active all year but exhibit aggressive behaviour and territoriality among coteries, even if members of adjacent coteries had previously split from the same coterie (Manno et al., 2007). Aggression between Cape ground squirrels is rare. Mongolian gerbils (*Meriones unguiculatus)* are social and live in kin groups of 2 – 18 individuals (Wang *et al.*, 2011; Deng et al. 2019). As the distance among individuals in different groups increases, the duration of the interactions decreases, suggesting a minimum distance for direct behavioural interactions to occur (Deng et al. 2019). Although interactions among Cape ground squirrel groups may be rare, it does not eliminate the possibility of kin benefits. Female Cape ground squirrels could still be interacting with neighbouring groups in other ways, hence the need for in-depth spatial analysis.

However, our second prediction, that Cape ground squirrels who have higher home range overlap with individuals of different groups would also be more highly related, was not supported. Resources can be represented by home range size, and the tolerance or sharing of these resources among groups may be suggestive of a kin-bias between groups (Armitage et al., 2011; Biondo et al., 2014; Podgórski et al., 2014; Price and Boutin, 1993; Stoen et al., 2005). Spatial resources can be incredibly important for female reproductive fitness and survival (Price and Boutin, 1993; Stoen et al., 2005; Viblanc et al., 2010; Viblanc et al., 2016). In yellow-bellied marmots, resources in the form of natal burrows are important in the formation of new groups (Armitage, 2009). Eastern grey kangaroos (*Macropus giganteus*) are a rare example of a group living species that does not display a dominance hierarchy or territoriality (Best et al., 2013). These kangaroos live in communities known as mobs (family groups), defined as a cluster of individuals that interact with each other more than others, and mobs are made up of adult and subadults females. Males are semi-solitary and roam around searching for mates, and subadult males will group by themselves. Increased home range overlap among grey kangaroo mobs was not correlated with relatedness but was a result of other factors, such as familiarity (Best et al., 2013). However, unlike Cape ground squirrels, home range overlap was high amongst grey kangaroo's mobs. In this population of Eastern grey kangaroo, food was abundant and large community overlap reflected an abundant food source as communities roamed freely and spatially overlapped with one another (Best et al., 2013). In contrast, the authors hypothesized that if resources were limited and patchy then the overlap of mob members would decrease (Best et al., 2013), similar to what is seen in Soay sheep (*Ovis aries*) in patchy habitats (Coulsont *et al.*, 1999). However, this is not the case for Cape ground squirrels.

Rain is highly correlated with resource availability for the regions inhabited by Cape ground squirrels (Pettitt et al. 2008). At a Namibian site with lower average rainfall (220 mm), female home range size was 3.2 ha (almost 3 times the home range in the current study) and home range size increased with increasing group size (Waterman, 1995; Pettitt et al. 2008). The group size of squirrels in Namibia may have been constrained by resources, as larger groups need larger feeding ranges to sustain all members as opposed to smaller groups (Waterman, 2002). Indeed, group splitting may have been due to resource competition (Waterman, 2002). Average rainfall at our study site was much higher (502 mm, Pettitt et al. 2008) and in 2017 rainfall was above average, suggesting that there was not a shortage of food. The average female home range size in the year of our study was much smaller than in the Namibia study, and there was no relationship between home range size and group size. In addition, squirrel density was lower in the low resource Namibian site (3.81 squirrels/ha) compared to our study site (Waterman, 1995; Manjerovic, 2010), suggesting that a smaller area in South Africa could sustain a greater number of squirrels. Certainly, burrow clusters in Namibia were further away from one another, and had a patchier distribution compared to our higher rainfall South African site, most likely because of the unpredictable rainfall in Namibia (Manjerovic, 2010). Furthermore, we did not find that home range overlap increased with relatedness, or that there was much home range overlap between squirrels of different family groups in comparison to the study in Namibia where squirrel groups overlapped by an average of 26% (Waterman, 1995). Cape ground squirrels experience reproductive suppression when groups get larger (Waterman, 2002; Pettitt and Waterman, 2011), and tend to split when groups become too large (Waterman, 2002; Chapter 2). Therefore, home range overlap could still incur some of these reproductive costs (Waterman, 2002), and as a consequence squirrel groups could remain spatially distinct in South Africa despite abundant resources.

We have also found that increased home range overlap between family groups did not contribute to increased adult female longevity or number of offspring in 2017. In Columbian ground squirrels (*Urocitellus columbianus*), proximity to kin reduced the time and energy breeding females devoted to territory defence because close kin were not as aggressive and less likely to take over neighboring territories of close kin (Viblanc et al., 2016). For Cape ground squirrels, they are not territorial, it is rare to interact among family groups, and aggressive behaviours both within and among family groups are extremely rare (Waterman, 1995; Pettitt and Waterman, 2011). In addition, our study was conducted during a high rainfall year and may not be reflective of years with lower rainfall, when resource competition might be more intense. However, we found that home range overlap within a family group was high and kin selection could still be occurring within groups.

Predation pressure is suggested to be a significant factor leading to sociality in Cape ground squirrels, as they are a small-bodied diurnal mammal living in an open habitat (Waterman, 1995; Waterman, 1997; Unck *et al.*, 2009; Cheney *et al.*, 2010; Edwards and Waterman, 2011). Increased distance to nearest neighbour increases vigilance in individual Cape ground squirrels (Unck *et al.*, 2009). The squirrels may still gain benefits of living near neighbouring kin, while still being spatially separated into adjacent groups, through alarm calling. In black-tailed prairie dogs, alarm calling was particularly nepotistic for females, benefiting non-descendant kin even when the females did not have any of their own offspring in the coterie (Hoogland, 1983). Belding's ground squirrels (*Spermophilus beldingi*) also have alarm calls that are kin directed (Sherman, 1985). We did not test kin biases in alarm calling, but the benefits of having other family groups near, even without spatial overlap, may aid in predator detection (Dunbar, 2018). In the future, studies on alarm calling, and spatial usage and distribution in Cape ground squirrels might reveal kin biases and impacts on survival.

In the Cape ground squirrel, we found an isolation by distance pattern which is consistent with previous research that suggests Cape ground squirrel kin tend to settle in adjacent groups (Chapter 2). The size and characteristic of Cape ground squirrel home range and distribution across the landscape may be indicative of resource availability, but we did not find kin biased pattern of home range overlap and relatedness between squirrel family groups. Nor did higher home range overlap contribute to greater per capita offspring, or longevity in female Cape ground squirrels. However, our study was conducted in a generally resource rich environment, in an above average rainfall year, which may have impacted our results. Overall, abundant resources may have interesting impacts on the manifestation of home range patterns in this social mammal. More research on kin selection between Cape ground squirrel family groups with differing resource levels is warranted.

References

Abercrombie, L. G., Anderson, C. M., Baldwin, B. G., Bang, I. C., Beldade, R., Bernardi, G., … Yuan, J. S. (2009). Permanent genetic resources added to molecular ecology resources database 1 january 2009-30 april 2009. *Molecular Ecology Resources*, *9*(5), 1375–1379. https://doi.org/10.1111/j.1755-0998.2009.02746.x

Altmann, J. (1974). Observational study of behavior: sampling methods. *Behaviour*, *49*(3–4), 227–266. https://doi.org/10.1163/156853974X00534

Armitage, K. B. (1981). Sociality as a life-history tactic of ground squirrels. *Oecologia*, *48*, 36–49.

Armitage, K. B. (1986). *Marmot polygyny revisited: determinants of male and female reproductive strategies* (D. I. Rubenstein and R. W. Wrangham, Eds.). Princeton University Press.

Armitage, K. B. (2009). Home range area and shape of yellow-bellied marmots. *Ethology Ecology & Evolution*, *21*(3–4), 195–207. https://doi.org/10.1080/08927014.2009.9522475

Armitage, K. B., Vuren, D. H. Van, Ozgul, A., and Oli, M. K. (2011). Proximate causes of natal dispersal in female Yellow-bellied marmots, Marmota flaviventris. *Ecology*, *92*(1), 218–227.

Arnaud, C. M., Dobson, F. S., and Murie, J. O. (2012). Philopatry and within-colony movements in Columbian ground squirrels. *Molecular Ecology*, *21*, 493–504. https://doi.org/10.1111/j.1365-294X.2011.05219.x

Best, E. C., Seddon, J. M., Dwyer, R. G., & Goldizen, A. W. (2013). Social preference influences female community structure in a population of wild eastern grey kangaroos. *Animal Behaviour*, *86*(5), 1031–1040. https://doi.org/10.1016/j.anbehav.2013.09.008

Biondo, C., Izar, P., Miyaki, C. Y., & Bussab, V. S. R. (2014). Social structure of collared peccaries (Pecari tajacu): Does relatedness matter? *Behavioural Processes*, *109*, 70–78. https://doi.org/10.1016/j.beproc.2014.08.018

Broquet, T., Ray, N., Petit, E., Fryxell, J. M., & Burel, F. (2006). Genetic isolation by distance and landscape connectivity in the American marten (Martes americana). *Landscape Ecology*, *21*(6), 877–889. https://doi.org/10.1007/s10980-005-5956-y

Calenge, C. (2006). The package adehabitat for the R software: tool for the analysis of space and habitat use by animals. *Ecological Modelling*, *197*, 516–519.

Cheney, D. L., Moscovice, L. R., Heesen, M., Mundry, R., & Seyfarth, R. M. (2010). Contingent cooperation between wild female baboons. *Proceedings of the National Academy of Sciences*, *107*(21), 9562–9566. https://doi.org/10.1073/pnas.1001862107

Chu, C., Henderson, D. J., & Parmeter, C. F. (2015). Plug-in Bandwidth Selection for Kernel Density Estimation with Discrete Data. *Econometrics*, *3*, 199–214. https://doi.org/10.3390/econometrics3020199

Clutton-Brock, T. H., & Lukas, D. (2012). The evolution of social philopatry and dispersal in female mammals. *Molecular Ecology*, *21*(3), 472–492. https://doi.org/10.1111/j.1365-294X.2011.05232.x

Coulsont, T., Albon, S., Pilkington, J., &Clutton-brock, T. I. M. (1999). Small-scale spatial dynamics in a fluctuating ungulate population. *Journal of Animal Ecology*, *68*(4), 658–671.

Deng, K., Wei, L., & Wang, D-H. (2019). Relatedness and spatial distance modulate intergroup interactions: experimental evidence from a social rodent. *Current Zoology*, *65*(5), 527–534. https://doi.org/10.1093/cz/zoy082

Dobson, F. S. (2013). The enduring question of sex-biased dispersal: Paul J. Greenwood’s (1980) seminal contribution. *Animal Behaviour*, *85*(2), 299–304. https://doi.org/10.1016/j.anbehav.2012.11.014

Dunbar, R. I. M. (2018). Social structure as a strategy to mitigate the costs of group living: a comparison of gelada and guereza monkeys. *Animal Behaviour*, *136*, 53–64. https://doi.org/10.1016/j.anbehav.2017.12.005

Edwards, A. W. F. (1974). The history of likelihood. *International Statistical Review*, 42(1), 9 – 15.

Edwards, S., & Waterman, J. M. (2011). Vigilance and grouping in the southern African ground squirrel (Xerus inauris). *African Journal of Ecology*, *49*(3), 286–291. https://doi.org/10.1111/j.1365-2028.2011.01262.x

Fournier, D. A., Skaug, H. J., Ancheta, J., Ianelli, J., Magnusson, A., Maunder, M., … Sibert, J. (2012). AD model builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optimal Methods Software*, *27*, 233–249.

Francisco, M. R., Gibbs, H. L., & Galetti, P. M. (2009). patterns of individual relatedness at blue manakin (Chiroxiphia caudata) leks. *The Auk*, *126*(1), 47–53. https://doi.org/10.1525/auk.2009.08030

Green, J. P., & Hatchwell, B. J. (2018). Inclusive fitness consequences of dispersal decisions in a cooperatively breeding bird, the long-tailed tit (Aegithalos caudatus). *Proceedings of the National Academy of Sciences*, *115*(47), 12011–12016. https://doi.org/10.1073/pnas.1815873115

Griffin, A. S., & West, S. A. (2002). Kin selection: fact and fiction. *Trends in Ecology & Evolution*, *17*(1), 15–21. https://doi.org/10.1016/S0169-5347(01)02355-2

Griffin, A. S. (2003). Kin discrimination and the benefit of helping in cooperatively breeding vertebrates. *Science*, *302*(5645), 634–636. https://doi.org/10.1126/science.1089402

Hamilton, W. D. (1964). The genetical evolution of social behaviour. II. *Journal of Theoretical Biology*, *7*(1), 17–52. https://doi.org/10.1016/0022-5193(64)90039-6

Hartig, F. (2016). *DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models*. Retrieved from https://cran.r-project.org/package=DHARMa

Harville, D. A. (1977). Maximum likelihood approaches to variance component estimation and to related problems. *Journal of the American Statistical Association*, *72*(358), 320–338. https://doi.org/10.1080/01621459.1977.10480998

Herzig-straschil, B. B. (1978). On the biology of xerus inauiris (Zimmermann, 1780) (Rodentia, Sciuridae). *Z. Säugetierkunde*, *43*, 262–278.

Hillegass, M. A., Waterman, J. M., & Roth, J. D. (2008). The influence of sex and sociality on parasite loads in an African ground squirrel. *Behavioral Ecology*, *19*(5), 1006–1011. https://doi.org/10.1093/beheco/arn070

Hoogland, J. L. (1983). Nepotism and alarm calling in the black-tailed prairie dog (Cynomys ludovicianus). *Animal Behaviour*, *31*(2), 472–479. https://doi.org/10.1016/S0003-3472(83)80068-2

Ishibashi, Y., Saitoh, T., Abe, S., & Yoshida, M. C. (1997). Sex-related spatial kin structure in a spring population of grey-sided voles Clethrionomys rufocanus as revealed by mitochondrial and microsatellite DNA analyses. *Molecular Ecology*, *6*(1), 63–71. https://doi.org/10.1046/j.1365-294X.1997.00152.x

Johannesen, E., Andreassen, H. P., & Ims, R. A. (2000). Spatial explicit demography: The effects habitat patch isolation have on vole matrilines. *Ecology Letters*, *3*(1), 48–57. https://doi.org/10.1046/j.1461-0248.2000.00119.x

King, W. J. (1989). Spacing of female kin in Columbian ground squirrels (Spermophilus columbianus). *Canadian Journal of Zoology*, *67*(1), 91–95. https://doi.org/10.1139/z89-014

Koprowski, J. L. (2002). Handling tree squirrels with a safe and efficient restraint. *Wildlife Society Bulletin*, *30*(1), 101–103.

Lambin, X., & Krebs, C. . J. (1993). Influence of female relatedness on the demography of Townsend’s vole populations in spring. *The Journal of Animal Ecology*, *62*(3), 536. https://doi.org/10.2307/5203

Laporte, F., and Mary-Huard, T. (2017). *Relatedness: Maximum Likelihood Estimation of Relatedness using EM Algorithm*. Retrieved from https://cran.r-project.org/package=Relatedness

Lukas, D., & Clutton-Brock, T. (2018). Social complexity and kinship in animal societies. *Ecology Letters*, *21*(8), 1129–1134. https://doi.org/10.1111/ele.13079

Maher, C. R. (2009). Genetic relatedness and space use in a behaviorally flexible species of marmot, the woodchuck (Marmota monax). *Behavioral Ecology and Sociobiology*, *63*(6), 857–868. https://doi.org/10.1007/s00265-009-0726-5

Maher, C. R. (2009). Genetic relatedness and space use in a behaviorally flexible species of marmot, the woodchuck (Marmota monax). *Behavioral Ecology and Sociobiology*, *63*(6), 857–868. https://doi.org/10.1007/s00265-009-0726-5

Manjerovic, M. B. (2010). *The Influence Of Sexual Selection On Behavioural And Physiological Mechanisms Underlying Reproductive Success In Male Cape Ground Squirrels (Xerus inauris)*. University of Central Florida.

Manjerovic, M. B., and Waterman, J. M. (2015). “Failure to launch”: is there a reproductive cost to males living at home? *Journal of Mammalogy*, *96*(1), 144–150. https://doi.org/10.1093/jmammal/gyu015

Manno, T. G., Dobson, F. S., Hoogland, J. L., and Foltz, D. W. (2007). Social group fission and gene dynamics among black-tailed prairie dogs (Cynomys ludovicianus) . *Journal of Mammalogy*, *88*(2), 448–456. https://doi.org/10.1644/06-mamm-a-099r1.1

Marshall, T. C., Slate, J., Krunk, L. E. B., and Pemberton, J. M. (1998). Statistical confidence for likelihood-based paternity. *Moleculular Ecology, 7*, 639–655.

Mateo, J. M. (2002). Kin-recognition abilities and nepotism as a function of sociality. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *269*(1492), 721–727. https://doi.org/10.1098/rspb.2001.1947

Michener, G. R. (1979). Spatial relationships and social organization of adult Richardson’s ground squirrels. *Canadian Journal of Zoology*, *57*(1), 125–139. https://doi.org/10.1139/z79-010

Mitchell, B. R. (2007). Comparison of programs for fixed kernel home range analysis. *Wildlife.Org*, *6*, 1–7. https://doi.org/10.1021/ct8002282

Pettitt, B. A., & Waterman, J. M. (2011). Reproductive delay in the female Cape ground squirrel (Xerus inauris). *Journal of Mammalogy, 92*(2), 378–386. https://doi.org/10.1644/10-MAMM-A-168.1

Pettitt, B. A., Waterman, J. M., & Wheaton, C. J. (2008). Assessing the effects of resource availability and parity on reproduction in female Cape ground squirrels: resources do not matter. *Journal of Zoology*, *276*(3), 291–298. https://doi.org/10.1111/j.1469-7998.2008.00491.x

Pew J., Muir P.H.,Wang J., Frasier T.R. (2015). related: an R package for analyzing pairwise relatedness from codominant molecular markers. *Molecular Ecology Resources*, 15: 557-561.

Podgórski, T., Lusseau, D., Scandura, M., Sönnichsen, L., & Jędrzejewska, B. (2014). Long-lasting, kin-directed female interactions in a spatially structured wild boar social network. *PLoS ONE*, *9*(6), e99875. https://doi.org/10.1371/journal.pone.0099875

Powers, S. T., & Lehmann, L. (2017). When is bigger better? The effects of group size on the evolution of helping behaviours. *Biological Reviews*, *92*(2), 902–920. https://doi.org/10.1111/brv.12260

Price, K., & Boutin, S. (1993). Territorial bequeathal by red squirrel mothers. *Behavioral Ecology*, *4*(2), 144–150. https://doi.org/10.1093/beheco/4.2.144

R Core Team. (2019). *R: A Language and Environment for Statistical Computing*. Retrieved from https://www.r-project.org/

Russell, A., Young, A., Spong, G., Jordan, N., & Clutton-Brock, T. H. (2007). Helpers increase the reproductive potential of offspring in cooperative meerkats. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1609), 513–520. https://doi.org/10.1098/rspb.2006.3698

Shave, J. R., and Waterman, J. M. (2017). The effects of familiarity and reproductive status on olfactory discrimination by female Cape ground squirrels (Xerus inauris). *Behavioural Ecology and Sociobiology*, *71*(12), 1–10. https://doi.org/10.1007/s00265-017-2410-5

Sherman, P. W. (1985). Alarm calls of Belding's ground squirrels to aerial predators: nepotism or self-preservation? *Behavioral Ecology and Sociobiology*, *17*(4), 313–323. https://doi.org/10.1007/BF00293209

Silk, J. B., Alberts, S. C., & Altmann, J. (2003). Social bonds of female baboons enhance infant survival. *Science*, *302*(5648), 1231–1234. https://doi.org/10.1126/science.1088580

Silk, J. B., Beehner, J. C., Bergman, T. J., Crockford, C., Engh, A. L., Moscovice, L. R., … Cheney, D. L. (2010). Strong and consistent social bonds enhance the longevity of female baboons. *Current Biology*, *20*(15), 1359–1361. https://doi.org/10.1016/j.cub.2010.05.067

Silverman, B.(1986). *Density Estimation for Statistics and Data Analysis* (Chapman an; Chapman and Hall, Eds.). London.

Skaug, H., Fornier, D., Bolker, B., Magnusson, A., and Nielson, A. (2016). *Generalized Linear Mixed Models using "AD Model Builder."*

Støen, O-G., Bellemain, E., Sæbø, S., & Swenson, J. E. (2005). Kin-related spatial structure in brown bears Ursus arctos. *Behavioral Ecology and Sociobiology*, *59*(2), 191–197. https://doi.org/10.1007/s00265-005-0024-9

Unck, C. E., Waterman, J. M., Verburgt, L., & Bateman, P. W. (2009). Quantity versus quality: how does level of predation threat affect Cape ground squirrel vigilance? *Animal Behaviour*, *78*(3), 625–632. https://doi.org/10.1016/j.anbehav.2009.05.028

Verdolin, J. L., and Slobodchikoff, C. N. (2002). Resources, not kinship, determine social patterning in the territorial Gunnison's prairie dog (Cynomys gunnisoni). *Ethology*, 59–69. https://doi.org/10.1111/j.1439-0310.2008.01590.x

Viblanc, V. A., Arnaud, C. M., Dobson, F. S., and Murie, J. O. (2010). Kin selection in Columbian ground squirrels (Urocitellus columbianus): littermate kin provide individual fitness benefits. *Proceedings of the Royal Society B: Biological Sciences*, *277*, 989–994. https://doi.org/10.1098/rspb.2009.1960

Viblanc, V. A., Saraux, C., Murie, J. O., Dobson, F. S., and Griffith, S. (2016). Kin effects on energy allocation in group-living ground squirrels. *Journal of Animal Ecology*, *85*(5), 1361–1369. https://doi.org/10.1111/1365-2656.12541

Walter, W. D., Fischer, J. W., Baruch-Mordo, S., and VerCauteren, K. C. (2011). *Modern Telemetry: What is the proper method to delineate home range of an animal using today's advanced GPS telemetry systems: The initial step* (O. Krejcar, Ed.). https://doi.org/10.5772/910

Wang, J. (2011). Coancestry: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Molecular Ecology Resources*, *11*(1), 141–145. https://doi.org/10.1111/j.1755-0998.2010.02885.x

Wang, Y., Liu, W., Wang, G., Wan, X., & Zhong, W. (2011). Home-range sizes of social groups of Mongolian gerbils Meriones unguiculatus. *Journal of Arid Environments*, *75*(2), 132–137. https://doi.org/10.1016/j.jaridenv.2010.08.008

Waterman, J. M. (1995). The social organization of the Cape ground squirrel (Xerus inauris; Rodentia: Sciuridae). *Ethology*, *101(2)*, 130–147.

Waterman, J. M. (1996). Reproductive biology of a tropical, non-hibernating ground squirrel. *Journal of Mammalogy*, *77*(1), 134–146.

Waterman, J. M. (1997). Why do male Cape ground squirrels live in groups? *Animal Behaviour*, *53*(4), 809–817. https://doi.org/10.1006/anbe.1996.0346

Waterman, J. M. (1998). Mating tactics of male Cape ground squirrels,Xerus inauris: consequences of year-round breeding. *Animal Behaviour*, *56*(2), 459–466. https://doi.org/10.1006/anbe.1998.0780

Waterman, J. M. (2002). Delayed maturity, group fission and the limits of group size in female Cape ground squirrels (Sciuridae: Xerus inauris). *Journal of Zoology*, *256(1)*, 113–120. https://doi.org/10.1017/S0952836902000146

Waterman, J. M., and Archibald, A. J. (2019). Both familiarity and kinship influence odour discrimination by females in a highly social African ground squirrel. *Animal Behaviour*, *148*, 145–151. https://doi.org/10.1016/j.anbehav.2018.12.007

Wright, S. (1943). Isolation by distance. *Genetics*, *28*(2), 114–138. https://doi.org/Article

Wrona, F. J., & Jamieson Dixon, R. W. (1991). Group size and predation risk: A field analysis of encounter and dilution effects. *The American Naturalist*, *137*(2), 186–201. https://doi.org/10.1086/285153

Zöttl, M., Fischer, S., & Taborsky, M. (2013). Partial brood care compensation by female breeders in response to experimental manipulation of alloparental care. *Animal Behaviour*, *85*(6), 1471–1478. https://doi.org/10.1016/j.anbehav.2013.03.045

Figures and Tables

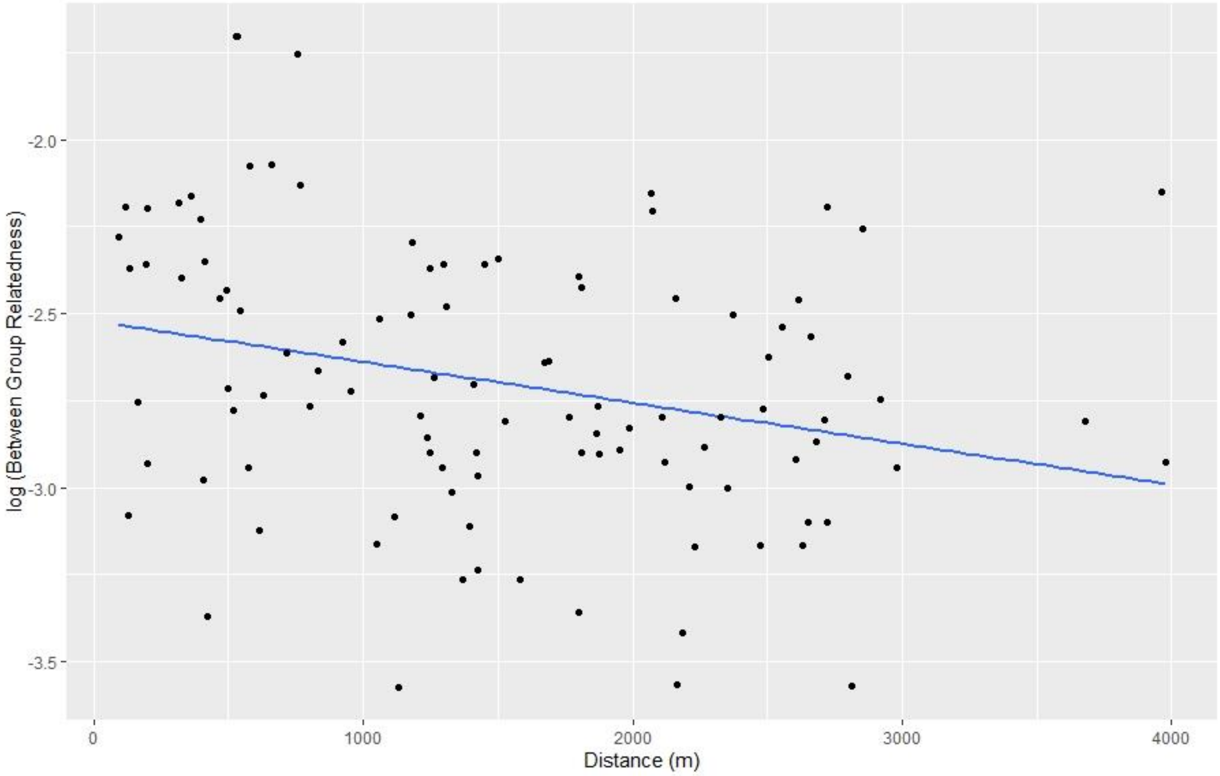

Figure 3.1: The negative relationship between distance (m) and average relatedness among family groups in Cape ground squirrels. LMM, estimate = -1.18e-04 ± 4.028e-06, t = -2.92, P = *0.0043, n = 105.*

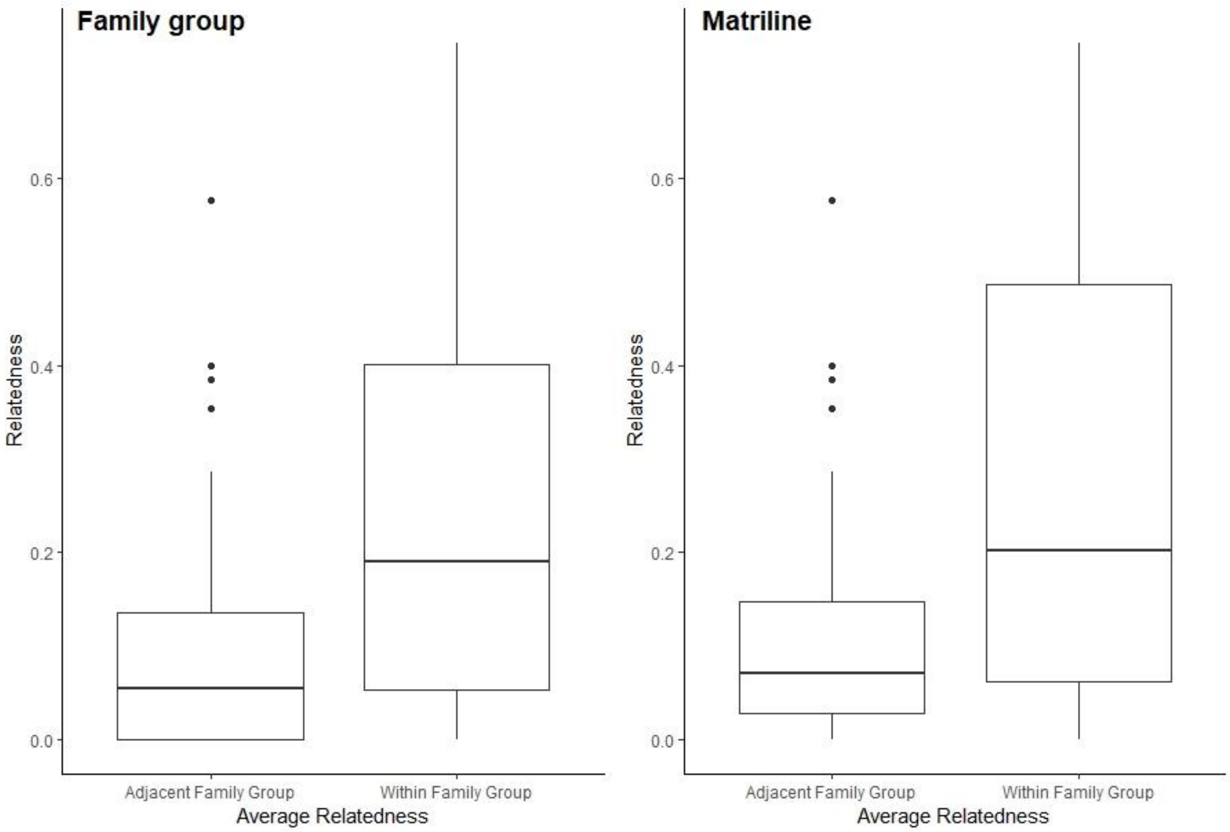

Figure 3.2: Average relatedness between adjacent family groups compared to relatedness within family groups excluding and including males in Cape ground squirrels. Males Included: Wilcoxon signed-rank test, W = 6351, $P < 0.0001$. Males excluded: Wilcoxon signed-rank test, W = 2841, $P < 0.0001$.

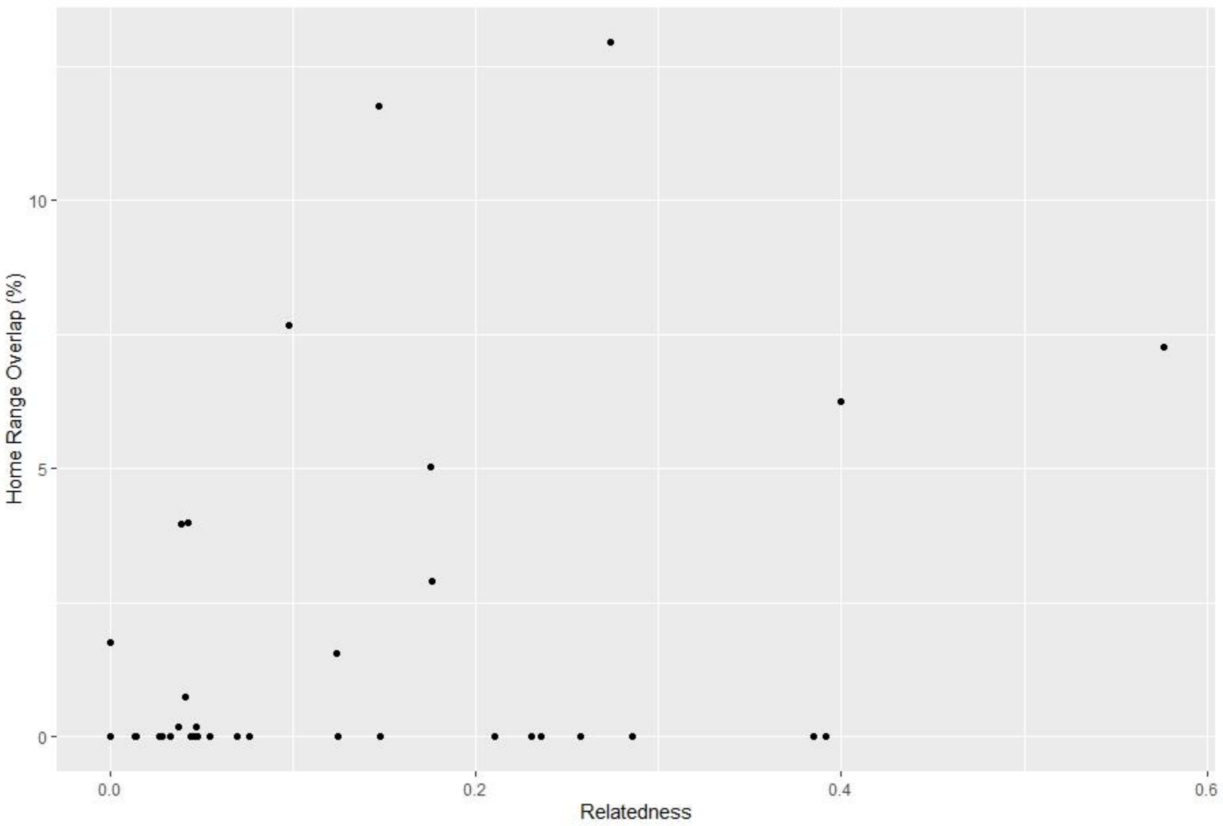

Figure 3.3: Relatedness of squirrel pairs among family groups and their home range overlap in Cape ground squirrels, beta GLMM, estimate = 0.31 ± 0.51, z-value = 0.6, *P* = 0.55, n = 46.

Chapter 4: Thesis Conclusions

The objective of our study was to explore patterns of relatedness and kin bias both within and among family groups of Cape ground squirrels and to understand how relatedness and kin bias influence the spatial distribution of squirrels. Within family groups, we have found promiscuity influenced the relatedness of social groups. We also found that direct fitness benefits are highly influential when squirrels leave the group, although fission can sometimes also include kin that are not offspring. Kin-biased proximity does not appear to factor into foraging behaviours away from the safety of the burrow cluster, most likely because predation pressure plays a significant influence in the spatial patterning and benefits of grouping in the squirrels. Among family groups, there was a distinct isolation by distance pattern, and low home range overlap. However, when tested, relatedness did not predict the percent of home range that squirrels shared either within or among social groups, nor did home range overlap predict survival or the per capita offspring of female squirrels. Home range alone was not influential in the spatial pattern of squirrels, but we did not incorporate predation pressure in our analysis of squirrels in a spatial context, which may be an important factor in their use space (Unck et al. 2009).

We have found that relatedness had a negative relationship with group size and declined over time when we considered the entire social groups. This result was consistent with what is found in promiscuous breeding systems in mammals (Aviles et al., 2004; Lukas et al.,2005). However, relatedness did not decline over time when we only examined matrilines. The relatedness of matrilines in Cape ground squirrels appears to be kept fairly high over time ($r > 0.25$) through the recruitment of daughters into the group and by the splitting of adult females to form groups with their offspring or close kin. Indeed, when group splitting occurred, female squirrels often maximized their direct fitness by splitting with their offspring. This pattern is consistent with what occurs in yellow-bellied marmots (*Marmota flaviventris*), where females will recruit daughters into their family group increasing matrilineal relatedness, and their direct fitness as daughters would help raise future offspring. For yellow-bellied marmots that are reproductively suppressed, they would leave the group to attempt to increase their direct fitness by leaving a group to start a new group with their subsequent daughters (Armitage, 1998; Armitage and Schwartz, 2000; Armitage et al., 2011). Immature Cape ground squirrel females can also experience reproductive suppression when they are in larger groups (Waterman, 2002) and it was the larger groups that split. For these immature females, group splitting to avoid reproductive suppression would allow them to maximize their own direct fitness through having offspring at an earlier age, which could increase their lifetime reproductive success.

We did not find that Cape ground squirrels had a kin bias in spatial proximity during foraging. Across many other small mammals, predation pressure is a significant reason why many animals form groups (Queller, 2016). Potentially, the reason for the lack of kin biased spatial proximity during foraging is simply due to the benefits of predation protection by living in a group. In this case, proximity to kin may not matter as all individuals in the group would gain direct fitness benefits through enhanced predator detection and alarm calling (Armitage, 1981). Evidence supports both the dilution effect and the many eyes hypotheses are the major benefit to grouping in Cape ground squirrels (Edwards and Waterman, 2011). In addition, for Cape ground squirrel females in the group, relatedness remains fairly stable over time (Chapter 2). Thus for females, they may use a rule of thumb that groupmates are kin and thus kin biases based on a fine degree of relatedness might be unnecessary. Ultimately, when a behaviour directly benefits all members of a group due to the increased number of individuals there is no reason why these individuals have to be kin (Kingma et al.,2014). This hypothesis is called group augmentation. Under this hypothesis, Cape ground squirrels are not predicted to be selective with whom they forage because all members of a group gain direct fitness benefits simply due to the increased number of individuals nearby enhancing predator detection and the dilution effect.

Among family groups, we did find isolation by distance, and that relatedness within a family group was higher than among family groups. Socially, female Cape ground squirrels are characterized by female kin groups (Waterman, 1995) and we found most groups settled near kin when group splitting occurred (Waterman, 2002). However, we did not find that among family groups, home range overlap corresponded to the relatedness of Cape ground squirrels, or that home range overlap influenced female squirrels in per capita offspring or increased survival. Therefore, the benefits of kin bias may not be detectable through the analysis of spatial resources in the form of home range in this population, or they may not be as important in female fitness (Kingma et al., 2014). In this study, Cape ground squirrels groups had very little home range overlap compared to previous studies in a site in Namibia that had lower rainfall and lower resources (Waterman, 1995). Previous studies have suggested that the South African study site is resource-rich in comparison to Namibia (Manjerovic, 2010; Pettitt et al., 2008). In Namibian populations of Cape ground squirrels feeding ranges increase with group size (Waterman, 1995), which suggests that resources are a limiting factor as squirrels needed more resources (larger area) to support larger groups. Indeed, in Namibia, squirrel home range overlap amongst groups may be due to the clumping and more patchy distribution of resources, and as a result, squirrels must share ranges between groups (Manjerovic, 2010; Pettitt et al., 2008; Waterman, 1995). In contrast, in our study, we did not find that home range size increased with increasing group size, suggesting that resources were not a limiting factor at our study site and/or were not clumped. Cape ground squirrel groups may remain spatially distinct, and do not need to share home ranges when resources are abundant. In addition, our research was conducted in an above-average rainfall year, suggesting that resources were more abundant. Future research on home range overlap within and among social groups should include low resource years as a comparison.

Sociality in Cape ground squirrels has been heavily influenced by predation pressure (Makenbach et al., 2013; Dunbar, 2018). However, we did not measure alarm calling and vigilance in conjunction with kin bias within a spatial context. In another ground-dwelling sciurid, the black-tailed prairie dog (*Cynomys ludovicianus*), individuals give alarm calls based on nepotism to warn genetic relatives (Hoogland, 1983). Despite the lack of overlap in the home range of Cape ground squirrel groups, alarm calls could be heard over distances that could reach nearby kin in neighboring groups, without the need for home range overlap. In this way, by remaining spatially close, Cape ground squirrels may continue to receive kin selected benefits in the context of predation protection but remain in separate family groups. Further research focused on nepotism and space use in the context of enhanced predator detection may address this hypothesis.

Female dispersal has been poorly documented in polyandrous species, where there are clear benefits to remaining philopatric. Indeed, the Cape ground squirrel is unique among sciurids in its social organization and spatial organization. Family groups do not show aggression towards each other, unlike other ground squirrels in North America (Van Der Marel et al., 2020) and most mammals. Overall kin selection and inclusive fitness benefits for Cape ground squirrels may not be as important as direct fitness benefits, although we also found some evidence of inclusive fitness when females split with close kin that were not their offspring. Future studies might delve into kin biased alarm calling or vigilance behaviour within a spatial context as predation is an important component to understanding the spatial structuring of Cape ground squirrel family groups. Further, social interactions, although rare between groups of Cape ground squirrels, are common within social groups. These interactions may prove important in the analysis of kin discrimination in this promiscuous cooperative breeding species.

References

Armitage, K. B. (1981). Sociality as a life-history tactic of ground squirrels. *Oecologia*, *48*, 36–49.

Armitage, K. B. (1998). Reproductive strategies of Yellow-bellied marmots: energy conservation and differences between the sexes. *Journal of Mammalogy*, *79*(2), 385–393. https://doi.org/10.2307/1382969

Armitage, K. B., & Schwartz, O. A. (2000). Social enhancement of fitness in yellow-bellied marmots. *Proceedings of the National Academy of Sciences*, *97*(22), 12149–12152. https://doi.org/10.1073/pnas.200196097

Armitage, K. B., Vuren, D. H. Van, Ozgul, A., & Oli, M. K. (2011). Proximate causes of natal dispersal in female yellow-bellied marmots, Marmota flaviventris. *Ecology*, 92(1), 218–227

Aviles, L., Fletcher, J. A., & Cutter, A. D. (2004). The kin composition of social groups: trading group size for degree of altruism. *The American Naturalist*, *164(2),* 132–144.

Edwards, S., & Waterman, J. M. (2011). Vigilance and grouping in the southern African ground squirrel (Xerus inauris). *African Journal of Ecology*, *49*(3), 286–291. https://doi.org/10.1111/j.1365-2028.2011.01262.x

Kingma, S. A., Santema, P., Taborsky, M., & Komdeur, J. (2014). Group augmentation and the evolution of cooperation. *Trends in Ecology & Evolution*, *29*(8), 476–484. https://doi.org/10.1016/j.tree.2014.05.013

Lukas, D., Reynolds, V., Boesch, C., & Viglilant, L. (2005). To what extent does living in a group mean living with kin? *Molecular Ecology*, *14*, 2181–2196. https://doi.org/10.1111/j.1365-294X.2005.02560.x

Manjerovic, M. B. (2010). *The Influence Of Sexual Selection On Behavioural And Physiological Mechanisms Underlying Reproductive Success In Male Cape Ground Squirrels (Xerus inauris)*. University of Central Florida.

Pettitt, B. A., Waterman, J. M., & Wheaton, C. J. (2008). Assessing the effects of resource availability and parity on reproduction in female Cape ground squirrels: resources do not matter. *Journal of Zoology*, *276*(3), 291–298. https://doi.org/10.1111/j.1469-7998.2008.00491.x

Queller, D. C. (2016). Kin selection and its discontents. *Philosophy of Science*, *83*(5), 861–872. https://doi.org/10.1086/687870

Unck, C. E., Waterman, J. M., Verburgt, L., & Bateman, P. W. (2009). Quantity versus quality: how does level of predation threat affect Cape ground squirrel vigilance? *Animal Behaviour*, *78*(3), 625–632. https://doi.org/10.1016/j.anbehav.2009.05.028

Van Der Marel, A., Waterman, J. M., & López-darias, M. (2020). Social organization in a North African ground squirrel. *Journal of Mammalogy*, *101*(3), 670–683. https://doi.org/10.1093/jmammal/gyaa031

Waterman, J. M. (1995). The Social organization of the Cape ground squirrel (*Xerus inauris*; Rodentia: Sciuridae). *Ethology*, *101*(2), 130–147.

Waterman, J. M. (2002). Delayed maturity, group fission and the limits of group size in female Cape ground squirrels (Sciuridae: Xerus inauris). *Journal of Zoology*, *256*(1), 113–120. https://doi.org/10.1017/S0952836902000146