Causes and consequences of inbreeding in the banded mongoose

David A. Wells

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Abstract

Inbreeding and inbreeding depression have been studied since the days of Darwin and yet we are still making important discoveries today. These discoveries can inform conservation practices as inbreeding depression can contribute to population extinction. Inbreeding is also an important subject in evolutionary biology as the selection pressures arising from inbreeding depression can profoundly shape the evolution of breeding behaviour. Several recent theoretical studies have argued that inbreeding avoidance should not always evolve and that because inbreeding increases the proportion of genes shared amongst relatives it can influence the evolution of altruism. In fact, current theory is rather at odds with the almost ubiquitous observation of inbreeding avoidance in empirical studies.

The banded mongoose (*Mungos mungo*) is a cooperative breeder which is unusual in that it frequently engages in incest. As part of the Banded Mongoose Research Project I have quantified the level of inbreeding and investigated both its causes and consequences. My study used 23 years of detailed behavioural observations on 1,956 individuals genotyped at 35-43 microsatellite loci. These genotypes were used to generate a nine-generation deep pedigree and inbreeding was estimated using the pedigree and microsatellite heterozygosity. Using generalised linear mixed models (GLMMs) I analysed the consequences of inbreeding using several measures of individual fitness and contributions to cooperative care. Finally I used piecewise structural equation modelling to investigate how changes in social variables influence individual breeding behaviour and ultimately cause inbreeding.

I found that incest is common in the banded mongoose despite severe inbreeding depression in numerous traits. This can be detected using both pedigree and genetic measures of inbreeding but combining both measures can provide the most explanatory power (**Chapter 2**). Inbreeding depression in juvenile survival can be reduced by offspring care, but offspring care itself suffers inbreeding depression which should oppose the evolution of closed inbred systems (**Chapter 3**). Finally, I show that breeding behaviour is adaptively adjusted according to the risk of inbreeding as the social environment changes (**Chapter 4**).

Understanding how inbreeding depression varies with genetic and environmental conditions is essential to explain the selection pressures that govern the evolution of mating behaviour. There is increasing awareness of the theoretical prediction that inbreeding depression does not inevitably lead to selection for inbreeding avoidance. In this thesis I find that inbreeding depression is not fixed but can be reduced by offspring care, which can potentially cause evolutionary feedback loops between inbreeding and care that have rarely been considered. Furthermore, inbreeding avoidance behaviour was not fixed but plastically adapted to environmental conditions which not only makes recording breeding behaviours more challenging but may also alter the selection pressures acting on them. In summary this thesis demonstrates the importance of several complexities of inbreeding behaviour in a wild population which must be more widely considered in order to fully understand the evolution of breeding behaviours.

Zusammenfassung

In dieser These erforsche ich die Ursachen und Konsequenzen von Inzucht in einem kooperativen Zuchttier, der Zebramunguste (*Mungos mungo*). Ich ermittelte, dass Inzucht in Zebramungusten, trotz starker Erbschäden in mehreren Merkmalen, verbreitet ist. Dies kann mit Abstammungsmerkmalen als auch genetischen Merkmalen von Inzucht festgestellt werden, aber eine Kombination beider Merkmale zusammen bietet die höchste Erklärungskraft (Kapitel 2). Erbschäden in den Überlebenschancen von jugendlichen Zebramungusten können durch Pflege der Jungtiere gemindert werden, jedoch erfährt diese Pflege an sich Erbschäden, welches die Evolution von geschlossenen ingezüchteten Systemen verhindern sollte (Kapitel 3). Schlussendlich weise ich nach, dass sich Fortpflanzungsverhalten adaptiv an das Risiko von Inzucht anpasst, wenn sich das soziale Umfeld ändert (Kapitel 4). Zusammenfassend demonstriert diese These die Bedeutung mehrerer Komplexitäten von Inzucht in einer Population wilder Zebramungusten. Diese Komplexitäten müssen stärker berücksichtigt werden um die Evolution von Zuchtverhalten vollends zu verstehen.

Summary for public engagement

The taboo against inbreeding is one of the “cultural universals” found across human populations (Rosman, Rubel, & Weisgrau, 2009). This taboo can be explained as a consequence of inbreeding depression because inbred individuals often have poor health (Charlesworth & Willis, 2009). In humans it is very difficult to estimate the magnitude of inbreeding depression because of conflated variables such as economic status (Bennett et al., 2002). However, estimates of inbreeding depression across a wide range of animals and plants have revealed that inbreeding depression can be severe enough to cause population extinctions (Frankham, 2005; O’Grady et al., 2006). As a result of inbreeding depression, many species have evolved inbreeding avoidance behaviours such as an aversion to breeding with individuals known from infancy, preferring to mate with individuals who are dissimilar to oneself in a variety of ways, or more simply dispersing away from your family (Pusey & Wolf, 1996).

The banded mongoose is an unusual species because inbreeding is common. Two thirds of individuals are inbred to some degree and 7% of individuals are at least as inbred as if their parents were full siblings (Wells, Cant, Nichols, & Hoffman, 2018). In this thesis, I investigate why the banded mongoose inbreeds more than similar species and identify how that affects both individuals and social groups. An obvious potential explanation of why this species might inbreed is that they do not suffer inbreeding depression. If this were true, understanding why could be an important step towards protecting endangered species, where inbreeding depression is often a serious concern. However, this was not the case.

I have found that banded mongooses inbreed despite substantial inbreeding depression. Very inbred males have less than a quarter of the number of offspring that outbred males have, and inbred pups are less likely to survive their first 90 days. However, offspring care is able to protect inbred pups so that they are just as likely to survive as any other pup. Some researchers have suggested that such an effect would mean there is no reason not to inbreed and so inbreeding should be very common when this effect is present (Avilés & Bukowski, 2005). I have also shown that inbred individuals rarely care for offspring, which should prevent inbreeding becoming very common, because if it were, offspring would receive little care and so inbred young would be unlikely to survive.

Why then does the banded mongoose inbreed despite these health costs? They are able to avoid inbreeding by breeding with mates from other social groups but they frequently do not. This is likely because rival groups are very aggressive to one and other, often killing adults and pups. I found that females were only likely to breed with rival groups when the risk of inbreeding in her own was high. Therefore, it seems that unless the risk of inbreeding is very high, the banded mongoose takes that risk in order to avoid violence from other groups. This implies that despite short-term benefits for the victors, violence between groups ultimately causes inbreeding and poor genetic health.

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Wells, D. A., Cant, M. A., Nichols, H. J., & Hoffman, J. I. (2018). A high-quality pedigree and genetic markers both reveal inbreeding depression for quality but not survival in a cooperative mammal. *Molecular Ecology*, *27*(9), 2271–2288.

Thesis structure

Abstract: A summary of the topic and major findings.

Summary for public engagement: A non-technical overview of the thesis aimed at generating interest amongst a broader audience.

Author contributions: A declaration of my contributions to the work in this thesis and agreement from my collaborators that this is true and that I may use this work in my thesis.

Publications: A list of publications arising directly from this thesis and from work conducted as part of this thesis.

Chapter 1, Thesis introduction: An overview of the inbreeding literature with special focus on the interactions between inbreeding and care as well as why inbreeding occurs. This section also introduces the study species and site.

Chapter 2, Inbreeding depression in the banded mongoose: Compares estimates of inbreeding depression in the banded mongoose based on two methods and presents statistics to incorporate both into a single model.

Chapter 3, Inbreeding depresses altruism in a cooperative society: Demonstrates that inbreeding depression can be mitigated by alloparental care. This chapter also finds that inbred individuals provide less alloparental care and as such inbreeding depression affects not only inbred individuals but the whole group.

Chapter 4, Extra-group breeding and the risk of inbreeding: Uses structural equation modelling to show how changes in self and social group influence breeding behaviour. Finds that costly inbreeding avoidance behaviour is only employed when the risk of inbreeding is high.

Chapter 5, Thesis discussion: Identifies four factors which should shape the selection pressures on inbreeding and considers their relative importance based on both the literature and results of this thesis.

Author contributions

This thesis is the product of international scientific collaboration. Below I detail my contributions and the contributions of my collaborators to the following chapters. All co-authors have seen this declaration and agreed that my contributions are correct and that I may include these chapters in my thesis.

Chapter 2

Wells, D. A., Cant, M. A., Nichols, H. J. *, & Hoffman, J. I.* (2018). A high-quality pedigree and genetic markers both reveal inbreeding depression for quality but not survival in a cooperative mammal. *Molecular Ecology*, 27(9), 2271–2288. *Joint senior authors.

J.I.H. and H.J.N. designed the research; D.A.W. genotyped individuals and assigned parentage and sibships; D.A.W. conducted data analyses with assistance from J.I.H.; J.I.H. and D.A.W. wrote the manuscript with comments from H.J.N.; field data were collected by H.J.N. and M.A.C. All of the authors read and commented upon the final manuscript.

Chapter 3

Wells, D. A., Cant, M. A., Hoffman, J. I.* & Nichols, H. J. * (*in prep.*). Inbreeding depresses altruism in a cooperative society. *Joint senior authors. This chapter was written with the intension of submitting it to the journal Science and is written following their formatting guidelines.

H.J.N. and J.I.H. designed the research. D.A.W. performed the data analysis and D.A.W. wrote the manuscript with comments from H.J.N., J.I.H. and M.A.C. Field data were collected as part of the long-term Banded Mongoose Research Project by H.J.N and M.A.C.

Chapter 4

Wells, D. A., Cant, M. A., Hoffman, J. I.* & Nichols, H. J. * (*in prep.*). Adaptive plasticity of extra-group breeding according to risk of inbreeding. *Joint senior authors. This chapter was written with the intention to submit to the journal Evolution and follows their formatting guidelines.

D.A.W, J.I.H, and H.J.N. designed the study; D.A.W. performed the data analysis and D.A.W and H.J.N wrote the manuscript with comments from J.I.H. Field data were collected as part of the long-term Banded Mongoose Research Project by H.J.N and M.A.C.

Prof. Michael A. Cant
College of Life and Environmental Sciences,
University of Exeter, Penryn, UK

Prof. Dr. Joseph I. Hoffman
Department of Animal Behaviour
University of Bielefeld, Germany

Dr. Hazel J. Nichols
Department of Biosciences
University of Swansea, UK
Department of Animal Behaviour
University of Bielefeld, Germany

Publications

Chapter 2 has been published during the course of my PhD.

Wells, D. A., Cant, M. A., Nichols, H. J. and Hoffman, J. I. (2018) 'A high-quality pedigree and genetic markers both reveal inbreeding depression for quality but not survival in a cooperative mammal', *Molecular Ecology*, 27, pp. 2271–2288.

I also contributed to the following papers which are not included in this thesis:

Mitchell, J., Vitikainen, E. I. K., **Wells, D. A.**, Cant, M. A. and Nichols, H. J. (2017) 'Heterozygosity but not inbreeding coefficient predicts parasite burdens in the banded mongoose', *Journal of Zoology*, 302(1), pp. 32–39. doi: 10.1111/jzo.12424.

Sheppard, C. E., Marshall, H. H., Inger, R., Thompson, F. J., Vitikainen, E. I. K., Barker, S., Nichols, H. J., **Wells, D. A.**, McDonald, R. A. and Cant, M. A. (2018) 'Decoupling of Genetic and Cultural Inheritance in a Wild Mammal', *Current Biology*. 28(11), p. 1846–1850. doi: 10.1016/j.cub.2018.05.001.

1

Thesis introduction

The genetic basis of inbreeding depression

Inbreeding is when two genetic relatives breed to produce an offspring which is said to be inbred. Inbred offspring frequently suffer inbreeding depression, which means they display lower fitness than an equivalent outbred individual (Hedrick & Garcia-Dorado, 2016). Darwin was among the first people to formally study this phenomenon, which he referred to as "the evil effects" (Darwin, 1876), and it continues to be an active area of research (for example Kardos & Shafer, 2018; Sandner & Diethart, 2016; Taylor et al., 2017). This research has revealed inbreeding depression across the animal and plant kingdoms (Crnokrak & Roff, 1999; Keller & Waller, 2002), where it imposes strong selection pressures and can contribute to population extinction (Frankham, 2005; O'Grady et al., 2006).

Inbreeding depression is caused by high levels of homozygosity; that is, carrying two copies of the same allele at many loci (Charlesworth & Willis, 2009; Kardos, Taylor, Ellegren, Luikart, & Allendorf, 2016). This occurs in inbred individuals because they may inherit the same allele from each parent when those parents are related. This homozygosity allows numerous deleterious recessive alleles to be expressed that would be masked in a heterozygote; it also limits the occurrence of overdominance where the heterozygous genotype is fitter than either homozygote (reviewed in Charlesworth & Willis, 2009).

In inbred individuals, the two identical copies are both inherited from a common ancestor and are said to be identical by decent (IBD). It is also possible for an outbred individual to inherit identical alleles which arose by separate mutations. These alleles are said to be identical by state; they are the same but have different origins. Although there is no functional difference in the fitness of these two types of homozygosity at a single locus, identity by state is a chance occurrence and likely to be relatively rare across the genome (Knief et al., 2015). In contrast, inbreeding can cause large continuous portions of the genome to be homozygous (Knief et al., 2015). Therefore, even when individual loci have only a small impact on fitness, they act collectively in inbred individuals to cause severe inbreeding depression (Charlesworth & Willis, 2009).

Measuring inbreeding

It is possible to estimate the proportion of an individual's genome that is IBD based on their ancestry, which can itself be inferred from a multigenerational pedigree. This proportion is the central measure of inbreeding and pedigree-based estimates have traditionally been the gold standard for measuring inbreeding (Pemberton, 2004). However, any errors in the pedigree can lead to inaccuracies in estimates of inbreeding (Reid et al., 2014; Taylor, Kardos, Ramstad, & Allendorf, 2015). Even when the pedigree is correct, it provides only the expected proportion of the genome which is IBD; the true value, however, varies due to recombination and Mendelian segregation (Hedrick, Kardos, Peterson, & Vucetich, 2016; Hill & Weir, 2011; Knief, Kempenaers, & Forstmeier, 2017).

Alternatively, it is possible to measure inbreeding directly using genome-wide measures of homozygosity. These methods are not without their own drawbacks and in general estimates based on microsatellites are less accurate than pedigree-based methods (Balloux, Amos, & Coulson, 2004; Slate et al., 2004). However, several studies have shown that measurements of homozygosity using very large panels of single nucleotide polymorphisms (SNPs) can be more accurate than pedigrees (Hoffman et al., 2014; Huisman, Kruuk, Ellis, Clutton-Brock, & Pemberton, 2016; Kardos et al., 2018; Kardos, Luikart, & Allendorf, 2015; J. Wang, 2016). For many studies, these large

SNP panels are still prohibitively expensive, but SNPs already play a growing role in genetic studies to more accurately measure inbreeding and identify putative fitness loci (Kardos et al., 2016).

Inbreeding depression is predominantly caused by the cumulative effect of many loci but some individual loci can have large effects (Charlesworth & Willis, 2009). Several studies which have measured inbreeding using heterozygosity have reported “local effects”, where heterozygosity at a single locus correlates with fitness. In some cases, these are taken as evidence of individual loci with large effects, but this view has been criticised on theoretical and statistical grounds (Szulkin, Bierne, & David, 2010). However, some diseases follow the rules of Mendelian inheritance implying that they are caused by a single recessive allele which has drifted to high frequency; for example lethal blindness in red-billed Choughs (Trask et al., 2016). For such cases it may be possible to identify the causal mutations using high-density arrays of SNPs or whole-genome resequencing (Kardos et al., 2016). These techniques may also identify genomic regions which contribute to inbreeding depression more generally (Kardos et al., 2018). However, it is an ongoing debate whether targeting specific fitness loci in conservation programmes will be beneficial or detrimental to a population’s genetic fitness (Kardos & Shafer, 2018).

Studying inbreeding depression in the wild can be complicated by the effect of environmental stressors. The fitness consequences of inbreeding are not constant across environments but become more severe under stressful conditions (Fox & Reed, 2011; Reed, Fox, Enders, & Kristensen, 2012). Conditions in the wild are often stressful and, as a result, inbreeding depression is generally less severe in captive or laboratory populations (Crnokrak & Roff, 1999; Hedrick & Garcia-Dorado, 2016). Therefore, estimates of inbreeding depression from studies on laboratory populations may not generalise well to wild populations where we are interested in the evolution of breeding behaviours

Inbreeding and care

Offspring care serves to protect offspring from environmental stresses such as harsh weather, starvation and pathogens (Royle, Smiseth, & Kölliker, 2012). Just as inbreeding depression is expected to be most severe under stressful conditions, it should be reduced under benign conditions. In keeping with this prediction inbreeding depression is reduced by offspring care in the burying beetle (Pilakouta, Jamieson, Moorad, & Smiseth, 2015). This protective effect is also evident in pre-natal care as Japanese quail selectively bred to lay large eggs (high prenatal investment) show less inbreeding depression in hatchling success than lines bred to lay small eggs (low prenatal investment) (Ihle, Hutter, & Tschirren, 2017).

If inbreeding depression is reduced, then selection for inbreeding avoidance will be weaker which may lead to a higher level of inbreeding tolerance in a population. (Avilés & Bukowski, 2005) suggest that a protective effect of group living completely prevented inbreeding depression in several spider species and thereby relaxed selection against inbreeding and enabled the repeated evolution of sociality in spiders. The social spiders form communal webs of hundreds of relatives that cooperate on different tasks such as web maintenance, hunting, and offspring care (Avilés, 1997). Each colony represents a separate lineage as there is no breeding between colonies, instead colonies form closed breeding systems where individuals only breed with relatives (Avilés & Purcell, 2012).

Despite the many well documented examples of offspring care from across the animal kingdom, reports of inbred mating systems are relatively rare (reviewed in Avilés & Purcell, 2012). Pilakouta et al. (2015) note that a protective effect of offspring care can only encourage high levels of inbreeding if care itself does not suffer from inbreeding depression. Otherwise, as inbreeding became common, offspring would stop receiving the care that protected them from inbreeding depression. Inbreeding depression for care has been reported in several species (García-Navas, Ortego, & Sanz, 2009; Pooley, Kennedy, & Nager, 2014). Inbred individuals might be expected to provide less care as they are less competitive and so have fewer resources to spare. Therefore, one reason that closed inbreeding systems may rarely evolve despite common care is that inbreeding depression for care itself acts as a breaking mechanism.

In contrast to other species, cooperative breeders might not be expected to display inbreeding depression in care. In cooperative breeders, there is often intense competition over a limited number of breeding spots and unsuccessful individuals can maximise their inclusive fitness by helping related breeders until they are able to secure their own breeding position (Hatchwell & Komdeur, 2000). If inbred individuals are unlikely to ever obtain a breeding position for themselves, their inclusive fitness may be best served by caring for the offspring of their relatives. There is mixed support for this idea; inbred meerkats provide more care (Nielsen, 2012), whereas El Oro parakeets with inbred helpers have lower fitness (Klauke, Segelbacher, & Schaefer, 2013).

Causes of inbreeding

Large, long-term studies provide convincing evidence that inbreeding depression incurs a substantial fitness cost (Reviews: Crnokrak & Roff, 1999; Keller & Waller, 2002; examples: Bérénos, Ellis, Pilkington, & Pemberton, 2016; Chen et al., 2016; Huisman et al., 2016; Nielsen et al., 2012; Reid et al., 2014; Sanderson, Wang, Vitikainen, Cant, & Nichols, 2015). This selection pressure has driven the evolution of inbreeding avoidance mechanisms in various species (Pusey & Wolf, 1996). Several important behaviours are recognised as potential mechanisms of inbreeding avoidance such as kin recognition, delayed maturation and dispersal (Pusey & Wolf, 1996). Despite the widespread selection pressure of inbreeding depression, we still observe inbreeding in wild populations (Nichols, 2017; Nichols, Cant, Hoffman, & Sanderson, 2014), many species show no evidence of inbreeding avoidance (Jamieson, Taylor, Tracy, Kokko, & Armstrong, 2009; Keller & Arcese, 1998; Reid et al., 2015), and a small number even show inbreeding preference (Kleven, Jacobsen, Robertson, & Lifjeld, 2005; Thünken, Bakker, Baldauf, & Kullmann, 2007; C. Wang & Lu, 2011).

Despite inbreeding depression, the evolution of inbreeding avoidance is not inevitable. There are several theoretical models which show that inbreeding may be selected for despite inbreeding depression (Duthie, Lee, & Reid, 2016; Kokko & Ots, 2006; Parker, 1979; Puurtinen, 2011; Smith, 1979). Kokko & Ots (2006) highlighted the disparity between predictions of inbreeding preference and a lack of empirical examples of inbreeding preference. This mismatch could, at least in part, stem from an assumption that inbreeding depression will inevitably select for inbreeding avoidance and as a result examples of inbreeding preference could have been under-tested and under-reported (Kokko & Ots, 2006; Szulkin, Stopher, Pemberton, & Reid, 2013). This section introduces several of the ideas used to explain why inbreeding avoidance may not evolve despite inbreeding depression.

It is only possible for mate choice to provide benefits if there are a range of mates to choose from. Intuitively there is little need for inbreeding avoidance when relatives are rarely encountered. Jamieson et al. (2009) extends this to show that the same is true when non-relatives are rare. In fact, they show that the fitness outcome of kin indiscriminate mating is only likely to be different from active choice when individuals frequently encounter relatives and non-relatives as potential mates. Without a meaningful range of potential mates, active choice can provide no benefits over random mating. Therefore, even if there is inbreeding depression, inbreeding avoidance behaviours will not be selected for unless there are both related and unrelated potential mates.

If inbreeding avoidance behaviours are costly, the benefits may not outweigh those costs (Waser, Peter, Austad, & Keane, 1986). It seems reasonable that inbreeding avoidance behaviours would be costly and although many costs have been proposed they are less often explicitly estimated (Forstmeier, Nakagawa, Griffith, & Kempenaers, 2014; Nichols, 2017). Delayed maturation obviously can incur a reduction in mating opportunities (Armitage & Downhower, 1974; Kokko & Ekman, 2002; O'Riain, Bennett, Brotherton, McIlrath, & Clutton-Brock, 2000; Van Vuren & Armitage, 1994). Similarly, dispersal has an energy cost and a possible risk to survival (Bonte et al., 2012; Clutton-Brock & Lukas, 2012). There is also the opportunity cost of rejecting a mate to consider; choosey individuals will have to spend more time and effort searching for an unrelated mate (Keller & Arcese, 1998). Importantly, these behaviours may still be selected for if their benefits outweigh these costs. However, simulations show that costs of mate choice can strongly limit the evolution of inbreeding avoidance (or preference) (Duthie & Reid, 2016; Kokko & Ots, 2006).

Breeding decisions therefore have to balance the benefits of producing high quality outbred offspring against the costs of inbreeding avoidance. These costs and benefits may not be fixed but may fluctuate with environmental conditions and species may be selected to adjust their breeding strategy accordingly (Annavi et al., 2014; Cohas, Yoccoz, Da Silva, Goossens, & Allainé, 2006). For example, in many species extra-pair breeding is more common when the risk of inbreeding is higher (Arct, Drobniak and Cichoń, 2015; but see Hsu *et al.*, 2015). Furthermore, breeding behaviour may be constrained away from optimality. In moose for example, inbreeding avoidance is hampered by skewed sex ratios and small population size (Herfindal et al., 2014).

It is worth noting that in theory inbreeding may provide inclusive fitness benefits by increasing the fecundity of relatives. If breeding with a male relative gives them an additional breeding opportunity it can increase the female's inclusive fitness too (Smith, 1979). Put another way, choosing to breed with a relative transmits more IBD alleles to the next generation because the offspring inherits IBD alleles from both the chooser and the related mate (Lehtonen & Kokko, 2015). To provide this benefit the inbred mating must be in addition to and not instead of an outbred mating, for example these benefits do not occur under strict monogamy (Lehtonen & Kokko, 2015). Although these theoretical benefits were first proposed decades ago (Fisher, 1941; Parker, 1979; Smith, 1979; Waser, Peter et al., 1986) they were ignored for many years by animal ecologists but have recently been highlighted and expanded on (Duthie et al., 2016; Duthie & Reid, 2016; Kokko & Ots, 2006; Lehmann & Perrin, 2003; Lehtonen & Kokko, 2015; Szulkin et al., 2013). Therefore, inbreeding may not be the result of constraints, costs, and a lack of choice; instead, inbreeding may occur because it provides benefits which outbreeding does not.

The banded mongoose

My study species is the banded mongoose *Mungos mungo*, a small (~1-2Kg) social carnivore. Individuals which survive their first year have an average life span of approximately 3.5 years, but the oldest individuals can survive to 12 years old (Cant, Nichols, Thompson, & Vitikainen, 2016). The rate of mortality is high (0.7) before individuals reach nutritional independence at 90 days as they are vulnerable to infanticide by rival groups and predators such as marabou storks *Leptoptilos crumeniferus* (Nichols, Cant, & Sanderson, 2015; Otali & Gilchrist, 2004). After 90 days mortality rates drop to 0.3 and continue to decrease before levelling off 0.1 after one year. Despite greater survival, adults are still vulnerable to rival groups, 10% of deaths with a known cause can be attributed to rival groups. Predators such as leopards *Panthera pardus* and monitor lizards *Varanus niloticus* are still a large threat to adults as they account for 51% of deaths with a known cause (Nichols et al., 2015; Otali & Gilchrist, 2004).

Banded mongooses live in social groups of ~20-30 adults, although groups of up to 75 individuals have been observed (Cant et al., 2016). Groups will forage in the morning and afternoon but rest in the shade during the hottest part of the day. The group stays together while foraging, usually within 10-20m of each other, but individuals aggressively defend their food from other group members (Cant, Vitikainen, & Nichols, 2013). Their diet consists primarily of insects but they will also eat fruit, eggs, and small vertebrates. Most prey items are found in dung, leaf litter, or buried a few inches in the soil; however, they will also scavenge from human rubbish opportunistically. Groups spend their nights in underground dens, often made in abandoned termite mounds or erosion gullies which are well covered by scrub. Within a territory, groups have 20-40 alternative den sites and usually sleep in a different den every 2-3 nights (Cant et al., 2016).

Banded mongooses are considered to be cooperative breeders because adults provide care to the pups of other group members, even when they have not bred themselves (Cant et al., 2013; Gilchrist & Russell, 2007; Hodge, 2007; Nichols, Amos, et al., 2012). However, it is important to note that the social organisation of the banded mongoose is unusual for a cooperative breeder. There is no dominant breeding individual or pair; instead, multiple males and females within a social group breed at the same time (Nichols, Amos, Cant, Bell, & Hodge, 2010). Females will start breeding from approximately ten months old (Gilchrist, Otali, & Mwanguhya, 2004). Females usually give birth to 2-4 pups and older females give birth to more pups than younger females (Nichols et al., 2010). Although males are capable of breeding from one year old they rarely breed successfully until three or four years old (Cant et al., 2016). All of the breeding females in a group come into oestrus within a week or so of each other and the older males in the group monopolise breeding opportunities by "mate-guarding" females in oestrus (Nichols et al., 2010). Mate-guarding entails closely following a female and chasing away other males which approach or attempt to mate. Although females can escape their mate-guard and breed with other males, the older males in a group father the majority of offspring through mate-guarding (Nichols et al., 2010). However, because multiple females come into oestrus at once no single male can father all of the group's offspring.

Despite conceiving on different days, the vast majority of females in a group give birth on a single night. This unusual birth synchronisation is believed to be a method of masking the maternity of pups and so avoiding infanticide by rival mothers (Cant, Nichols, Johnstone, & Hodge, 2014; Hodge, Bell, & Cant, 2011). This striking level of breeding synchrony only occurs within groups, not across the population.

For their first month, pups remain in the group's underground den while adults go on foraging expeditions. Although hidden from sight, if the litter of pups is left unattended they are vulnerable to predators and infanticide by rival banded mongoose groups (Cant et al., 2016). In order to protect the group's litter, one, two or sometimes more adults will forgo a foraging opportunity and remain at the den in a behaviour known as babysitting (Cant, 2003; Hodge, 2007). When litters are consistently left without babysitters it is very rare that any of the pups survive (Cant, 2003). Contributions to babysitting are not limited to the parents of pups. In fact, babysitting is primarily performed by young males which rarely breed (Cant, 2003; Hodge, 2007).

At one month old pups emerge from the den and accompany adults on foraging expeditions. However, they still receive care from older group members. Pups beg for food from foraging adults and many form a one-to-one caring relationship with an adult who is termed their escort (Cant et al., 2016). Escorts provide food and grooming for pups as well as carrying them to safety when predators threaten (Hodge, 2005). As with babysitting, it is not only parents which escort pups and escort care is not specifically directed towards related individuals (Vitikainen et al., 2017).

Females will breed up to four times per year and conceive shortly after giving birth (Cant, 2000; Cant et al., 2013). Females provide less care than males because any resources they invest in care necessarily means fewer resources to invest in their next litter (Gilchrist & Russell, 2007; Hodge, 2007; Nichols, Amos, et al., 2012). Females which are heavier at conception give birth to larger, more competitive offspring which are more likely to survive to independence (Hodge et al., 2009). In contrast, male reproduction is not strongly affected by body mass, instead it depends on an age-based dominance hierarchy so resources invested in care do not greatly reduce a male's reproduction (Hodge, 2007; Nichols, Amos, et al., 2012; Nichols et al., 2010). However, evidence suggests that older males moderate their care to minimise its impact on their reproduction. For example they rarely babysit whilst females are in oestrus (Cant, 2003; Hodge, 2007) which is approximately 10 days after giving birth (Cant, 2000).

Unlike the majority of other cooperative breeders, most banded mongooses remain in their natal group for their entire lives (Nichols, Jordan, Jamie, Cant, & Hoffman, 2012). What makes the banded mongoose especially interesting from an inbreeding perspective is that most individuals breed with other members of their natal group, which frequently contains close relatives (Nichols et al., 2014, 2015). This contrasts with many other cooperative breeders where sex-biased dispersal or extra-group breeding commonly reduce the risk of inbreeding (Nichols, 2017). When females breed within their group they breed with close relatives less often than expected by chance (Sanderson et al., 2015), but within-group breeding commonly leads to highly inbred individuals despite this limited inbreeding avoidance (Nichols et al., 2014, 2015). Females can successfully avoid inbreeding by mating with extra-group males; however, it is fairly uncommon as only 18% of pups are sired by extra-group males (Nichols et al., 2015).

Although the majority of individuals stay in their natal pack for their entire life, some are evicted and disperse to form new groups (Cant et al., 2013). Evictions occur when resource competition within groups is high, with several individuals being evicted together (Thompson et al., 2016). The evictees are usually female but sometimes males are also evicted alongside them. The evicted individuals form single sex dispersing cohorts, even when females and males are evicted simultaneously (Cant, Otali, & Mwanguhya, 2001). These cohorts can form new groups either by joining up with a dispersing cohort of the opposite sex to establish a new territory, or by displacing the same sex individuals of a rival group (Thompson et al., 2016; Thompson, Marshall, Vitikainen, Young, & Cant, 2017). This is the principle way by which new groups are formed and it has important implications for their inbreeding. When groups are formed the opposite sex members are unrelated, although same sex individuals may be related (Nichols, Jordan, et al., 2012). There is initially no risk of inbreeding when mating within groups; however, over time both relatedness and the risk of inbreeding increase due to male and female philopatry.

For this thesis, I used data gathered from a study population of banded mongooses on Mweya Peninsula, Queen Elizabeth National Park, Uganda (0°12´S, 27°54´E). This population has been under continuous study as part of the Banded Mongoose Research Project since 1995 and all of the banded mongoose studies referred to above were conducted on this population. It was a partially closed population as it was mostly surrounded by lake Edward and the Kazinga channel, although there was some immigration and emigration from the adjoining mainland. Over the course of the study over 100 individuals have emigrated from the study site and over 200 individuals not born on Mweya were observed at the study site. However, these immigrants did not necessarily settle in Mweya, some were driven out by resident groups (Thompson et al., 2017). The population density was relatively high on Mweya (18 individuals Km^{-2}) compared to the Serengeti plains (3 individuals Km^{-2}, Cant et al., 2013). However, mortality rates on Mweya were similar to those observed in the Rwenzori national park, Uganda (Rood, 1975).

All individuals in the population had some form of permanent identification, early in the study it was a unique tattoo but latterly a subcutaneous pit tag was used (TAG-P-122IJ, Wyre Micro Design Ltd., UK). Individuals were also recognisable on sight due to shave patterns on adults and dye patterns in juveniles. One or two individuals within each social group were fitted with a 27-g radio collar (<2% of body mass, Sirtrack Ltd., New Zealand) and a 20-cm whip antenna (Biotrack Ltd., UK). This allowed the groups to be tracked and visited every two to four days in order to record detailed behavioural and life history data. Further details of the study methods are provided in each chapter.

Research objectives

In chapter 2 my objective was to compare the power of pedigree and microsatellite measures of inbreeding to explain variation in fitness. In order to assign parentage, I genotyped 192 individuals at 35 microsatellite loci and combined these genotypes with existing behavioural and genetic data on a further 1,748 individuals. This allowed me to update the existing Banded Mongoose Research Project 9-generation pedigree (Sanderson et al., 2015) and calculate individuals' inbreeding coefficient. I also calculated a genetic measure of inbreeding as the standardised multi-locus heterozygosity (sMLH) of the microsatellite loci. I modelled juvenile and adult survival and "quality" using both inbreeding measures separately which allowed me to assess their relative ability to measure inbreeding. "Residual regression" is a technique which can combine both estimates and I demonstrate that in some cases this combination can explain more variation in fitness than either single measure.

In chapter 3 my aim was to explore the relationship between inbreeding and offspring care. I found that escort care protects inbred pups from inbreeding depression in juvenile survival. However, this care was not preferentially directed towards inbred pups even though they benefit from it more than outbred pups. I also show that inbred individuals provide less care; therefore, being inbred can have fitness consequences for other outbred members of the group.

The goal of chapter 4 was to explore how individual female banded mongooses adjust their extra-group breeding behaviour with social and environmental conditions. Within-group breeding often results in inbreeding whereas females are commonly unrelated to their extra-group mates (Nichols et al., 2014). Despite this, the majority of females conceive to within-group males which commonly results in inbreeding (Nichols et al., 2015). I used piecewise structural equation modelling to investigate changes in social groups and their effect on breeding behaviour and the inbreeding of resulting offspring. I found that the risk of inbreeding increases over time and that in response females are more likely to breed with extra-group males, which in-turn produces outbred offspring.

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A high-quality pedigree and genetic markers both reveal inbreeding depression for quality but not survival in a cooperative mammal

David A. Wells, Michael A. Cant, Hazel J. Nichols* & Joseph I. Hoffman*

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Abstract

Inbreeding depression, the reduced fitness of offspring of closely related parents, is commonplace in both captive and wild populations and has important consequences for conservation and mating system evolution. However, because of the difficulty of collecting pedigree and life history data from wild populations, relatively few studies have been able to compare inbreeding depression for traits at different points in the life cycle. Moreover, pedigrees give the expected proportion of the genome that is identical by descent (IBD_g) whereas in theory with enough molecular markers realised IBD_g can be quantified directly. We therefore investigated inbreeding depression for multiple life-history traits in a wild population of banded mongooses using pedigree-based inbreeding coefficients (f_{ped}) and standardised multilocus heterozygosity (sMLH) measured at 35–43 microsatellites. Within an information theoretic framework, we evaluated support for either f_{ped} or sMLH as inbreeding terms and used sequential regression to determine whether the residuals of sMLH on f_{ped} explain fitness variation above and beyond f_{ped}. We found no evidence of inbreeding depression for survival, either before or after nutritional independence. By contrast, inbreeding was negatively associated with two quality related traits, yearling body mass and annual male reproductive success. Yearling body mass was associated with f_{ped} but not sMLH, while male annual reproductive success was best explained by both f_{ped} and residual sMLH. Thus, our study not only uncovers variation in the extent to which different traits show inbreeding depression, but also reveals trait-specific differences in the ability of pedigrees and molecular markers to explain fitness variation and suggests that for certain traits genetic markers may capture variation in realised IBD_g above and beyond the pedigree expectation.

Introduction

Inbreeding depression, the reduction in offspring fitness that can result from incestuous matings, occurs in a wide range of both captive and wild populations (Hedrick & Garcia-Dorado, 2016; Keller & Waller, 2002). Inbreeding increases the proportion of the genome that is identical by descent (IBD_g), which in turn reduces fitness mainly through the increased expression of deleterious recessive alleles but also due to increased homozygosity at loci showing overdominance (Charlesworth & Willis, 2009). The resulting loss of fitness can be substantial and is believed to have shaped the evolution of dispersal and mating behaviour in many species. Consequently, quantifying the severity of inbreeding depression in natural populations is essential for understanding population and evolutionary dynamics (Hedrick & Garcia-Dorado, 2016; Keller & Waller, 2002; Nichols, 2017; Szulkin, Stopher, Pemberton, & Reid, 2013).

Inbreeding depression is predicted to be strongest for traits that are closely related to fitness such as survival and reproduction, as these will be subject to stronger directional selection and therefore exhibit greater directional dominance (Falconer & Mackay, 1996). This is supported by a meta-analysis of 54 animal species, although most of the studies involved were of captive or experimental populations (DeRose & Roff, 1999). However, understanding how inbreeding depression affects different life history traits in natural populations is more challenging due to the difficulty of collecting high-quality lifetime fitness measures and generating deep, well resolved pedigrees. Furthermore, strong viability selection against inbred offspring will result in an adult population in which inbred individuals are rare, potentially making it more difficult to detect inbreeding depression for late acting traits (Huisman, Kruuk, Ellis, Clutton-Brock, & Pemberton, 2016).

Traditionally, pedigrees were considered the gold standard for measuring inbreeding in natural populations (Pemberton, 2004). However, the vast majority of pedigrees are incomplete and will also contain errors that can impair their ability to detect inbreeding depression (Reid et al., 2014; H. R. Taylor, Kardos, Ramstad, & Allendorf, 2015). Additionally, pedigrees cannot account for inbreeding caused by ancestors who are not included in the pedigree. This can result in downwardly biased estimates of inbreeding, particularly where the pedigree is only a few generations deep and relationships among the founders are unknown (Kardos, Luikart, & Allendorf, 2015). Arguably, an even greater issue is that pedigrees simply cannot be generated for the majority of wild populations, many of which are large and demographically open.

A further drawback of pedigrees is that, even when multiple generations of accurate ancestry data can be collected, the pedigree inbreeding coefficient (f_{ped}) quantifies an individual's *expected* $\mathrm{IBD_g}$ based on the known common ancestors of its parents, whereas *realised* $\mathrm{IBD_g}$ will differ stochastically from this expectation due to Mendelian segregation and recombination (Hedrick, Kardos, Peterson, & Vucetich, 2016; Hill & Weir, 2011; Knief, Kempenaers, & Forstmeier, 2017). The variance in realised $\mathrm{IBD_g}$ among individuals with the same f_{ped} will be higher for species with few chromosomes and short genetic maps (Fisher, 1965; Franklin, 1977; Hill & Weir, 2011; Kardos et al., 2015) and will also decrease with the number of generations separating an inbred individual from its common parental ancestor(s) as IBD chromosomal segments are gradually broken down by successive recombination events (Hedrick et al., 2016).

As deep, high-quality pedigrees are also lacking for the majority of natural populations, many studies have used the heterozygosity of small panels of typically around 10–20 presumed neutral markers such as microsatellites as a surrogate measure of $\mathrm{IBD_g}$. The result is a large and expanding literature describing heterozygosity-fitness correlations (HFCs) covering a long list of traits and species (Chapman, Nakagawa, Coltman, Slate, & Sheldon, 2009). However, estimates of $\mathrm{IBD_g}$ based on such small panels of markers will tend to have limited precision due to both high sampling variance and the difficulty of distinguishing identity by descent (IBD) from identity by state (IBS, Balloux, Amos, & Coulson, 2004; Slate et al., 2004). Recent simulation and empirical studies suggest that these issues can be overcome with very large panels of markers, with around ten thousand or more single nucleotide polymorphisms (SNPs) being preferable under most circumstances even to a deep pedigree for quantifying inbreeding depression (Hoffman et al., 2014; Huisman et al., 2016; Kardos et al., 2015; Wang, 2016). However, until SNP genotyping costs fall to the point where such large datasets can be collected within the budgets of most projects, it is likely that microsatellites will continue to be used to investigate inbreeding effects in wild populations.

Only a handful of studies have directly compared the ability of f_{ped} and microsatellites to detect inbreeding depression (e.g. Grueber, Waters, & Jamieson, 2011; S. S. Taylor et al., 2010), and these have uncovered mixed results. At one end of the spectrum, Nietlisbach et al. (2017) used an unusually deep and well resolved song sparrow pedigree to show that f_{ped} outperformed microsatellite heterozygosity, even when the latter could be calculated from an unusually large panel of 160 markers. At the other end, both Forstmeier et al. (2012) and Hammerly et al. (2013) found that smaller panels of around ten microsatellites explained more fitness variation than f_{ped}. These contradictory outcomes probably reflect a multitude of factors including variation among studies in pedigree depth and quality, marker number and resolution, as well as factors intrinsic to a given system such as the recombination landscape.

Consequently, in order to obtain a more general picture of how pedigrees and genetic markers can capture fitness variation, similar studies of a wider variety of taxa are needed.

A related question is whether the heterozygosity of genetic markers can explain fitness variation above and beyond that explained by f_{ped}. Some studies have approached this question by testing for HFCs within individuals of the same pedigree inbreeding class (Hansson, Westerdahl, Hasselquist, Åkesson, & Bensch, 2004; Hemmings, Slate, & Birkhead, 2012), while others have constructed statistical models of the focal traits containing both f_{ped} and marker heterozygosity (e.g. Bensch et al., 2006), an approach that Nietlisbach et al. (2017) recently termed 'residual heterozygosity-fitness correlation'. However, if these two inbreeding measures are strongly correlated, the variance explained by either term cannot be properly partitioned due to collinearity (Dormann et al., 2013). One way to account for this would be to take the residuals of marker heterozygosity on f_{ped} and fit this as an explanatory variable alongside f_{ped}. The variance shared by these two terms will be attributed to the pedigree, while any effect of residual heterozygosity will reflect the ability of the markers to detect variation in realised IBD_{g} that cannot be captured by the pedigree. This approach is known as 'sequential regression' (Graham, 2003) or sometimes 'residual regression' and has been shown to perform well in a comparison of approaches for dealing with collinearity (Dormann et al., 2013).

A long term study of banded mongooses (*Mungos mungo*) provides an excellent opportunity to investigate the strength of inbreeding depression for multiple traits, as well as to explore the ability of f_{ped} and marker heterozygosity to capture fitness variation in a wild vertebrate population. Banded mongooses live in social groups of 10–40 adults and, unlike most cooperative breeders, members of both sexes habitually breed within their natal pack despite the presence of close relatives (Nichols, Cant, Hoffman, & Sanderson, 2014). As a result, inbreeding appears to be common despite evidence that females attempt to avoid inbreeding and that males preferentially mate guard more distant relatives (Sanderson, Wang, Vitikainen, Cant, & Nichols, 2015). Furthermore, inbreeding appears to have fitness implications for offspring as recent studies have uncovered inbreeding depression for both yearling body mass and parasite load (Mitchell, Vitikainen, Wells, Cant, & Nichols, 2017; Sanderson et al., 2015). However, although both of these studies were based on a high-quality, nine-generation deep pedigree, only the latter compared the ability of f_{ped} and microsatellite heterozygosity to detect inbreeding depression.

Here, we genotyped an additional 192 individuals at 35 microsatellite loci in order to enlarge the existing banded mongoose pedigree to include 777 individuals with all four grandparents known. The resulting dataset was then used to investigate inbreeding depression for a variety of traits acting at different time points in the life cycle: (i) survival to nutritional independence; (ii) survival beyond nutritional independence; (iii) yearling body mass; and (iv) annual reproductive success. We additionally evaluated the abilities of f_{ped}, marker heterozygosity and residual marker heterozygosity to detect inbreeding depression. We hypothesised that viability selection against inbred individuals would reduce both the mean and variance in inbreeding in the adult population, thereby rendering inbreeding depression for late-acting traits more difficult to detect. We also hypothesised that, despite having a high-quality pedigree, our moderately large panel of microsatellites would allow us to explain fitness variation above and beyond that explained by f_{ped}, and that the explanatory power of the markers would increase with the number of loci.

Materials and methods

Study site, individual identification and sample collection

This study was conducted on a free-ranging population of banded mongooses in Queen Elizabeth National Park, Uganda (0°12'S, 27°54'E). The study area comprises approximately 10 km^2 of savannah on and around the Mweya Peninsula and a weather station near the centre measures the amount of daily rainfall. Genetic, behavioural and life-history data were collected from a total of 1,978 individuals between May 1997 and July 2016 inclusive. At any one time, the population consisted of approximately 250 individuals belonging to 10–12 social groups. A combination of approaches were used to identify individuals in the field. The majority of individuals were first captured as pups and given either a unique tattoo or a subcutaneous pit tag (TAG-P-122IJ, Wyre Micro Design Ltd., UK) to allow permanent identification. For genetic analysis, a 2mm tissue sample was taken from the tip of the tail using surgical scissors and a dilute solution of potassium permanganate was applied to minimise infection risk. To identify individual mongooses by sight, commercially available hair dye (L'Oreal, UK) was used to apply unique patterns to animals up to six months of age. Adults were given a unique shave pattern and, after they had stopped growing, were fitted with colour-coded plastic collars. To maintain dye markings, shave patterns and collars, all individuals were trapped every 3–6 months as described by Cant (2000), Hodge (2007) and Jordan et al. (2010).

Life history data collection

Detailed behavioural and life history data were collected by visiting each pack every 2–4 days. All individuals in the population were habituated to human observers. Mongoose packs could be reliably located because one or two adults in each pack were fitted with a 27g radio collar ($<2\%$ of body mass, Sirtrack

Ltd., New Zealand) with a 20cm whip antenna (Biotrack Ltd., UK). Age could be determined for the majority of individuals born within the study site based on their mother's parturition dates, but was unknown for immigrants. Individual lifespan was calculated as the time in days between the date of birth and the date of death. Death could be distinguished from dispersal because mongooses disperse in groups (Cant, Otali, & Mwanguhya, 2001) and dispersal events are also generally preceded by a period of aggression from the rest of the group (Thompson et al., 2016).

Escorting is a form of care unique to banded mongooses that affects offspring fitness (Cant, Vitikainen, & Nichols, 2013; Gilchrist, 2004; Hodge, 2005). Escorting begins approximately 27 days after birth, when pups leave the den and begin to forage with the pack (Gilchrist, 2004). During this time, some of the pups form an exclusive one-to-one relationship with an adult who feeds, grooms, carries, and protects them from predators. We therefore collected detailed data on escorting behaviour so that we could incorporate escorting into our analyses of early-acting fitness traits. Throughout the escorting period, which lasts approximately two months, we visited packs once or twice daily. If an adult was closely associated with a pup (i.e. spent more than half of a 20 minute observation period within 0.5m of the focal pup) the adult was deemed to be an escort for that pup. For each pup, we quantified the amount of care received as the proportion of visits during which a pup was seen with an escort.

Ethical statement

Research was carried out under licence from the Uganda National Council for Science and Technology and all procedures were approved by the Uganda Wildlife Authority. All research procedures adhered to the ASAB Guidelines for the Treatment of Animals in Behavioural Research and Teaching and were approved by the Ethical Review Committee of the University of Exeter. Our trapping procedure has been used over 8000 times and tissue samples have been taken from over 1900 individuals with no adverse effects.

DNA extraction and microsatellite genotyping

Prior to this study, genetic data were available for 1,748 individuals that were tissue sampled between 1997 and 2013 and genotyped at up to 43 microsatellite loci (Sanderson et al. 2015). All of these loci are known to be in Hardy-Weinberg and linkage equilibrium in the study population (Sanderson et al. 2015). To enlarge this dataset, we genotyped an additional 192 individuals that were sampled between 2014 and 2015 at 35 of these microsatellites. We excluded 8 loci that had previously been amplified individually and visualised through radioactive incorporation but which failed to amplify reliably in multiplexed PCRs using fluorescent labelled primers. DNA was extracted using Qiagen® DNeasy blood and tissue kits following the manufacturer's protocol. The genotyping was conducted as described in detail by Sanderson et al. (2015). Briefly, fluorescently labelled microsatellite primers were incorporated into seven separate multiplexes. PCR reactions were conducted using a Type It kit (Qiagen) according to the manufacturer's protocol with an annealing temperature of 57ºC and a reaction volume of 12µl. PCR products were resolved by electrophoresis on an ABI 3730xl capillary sequencer and allele sizes were scored using GeneMarker version 1.95 (SoftGenetics, Pennsylvania, USA).

Pedigree construction

The resulting microsatellite dataset was used to update an existing banded mongoose pedigree, comprising 1,748 individuals genotyped at 35–43 microsatellite loci (Sanderson et al., 2015). We followed the protocol of Sanderson et al. (2015) to extend the pedigree using a combination of

MasterBayes (Hadfield, Richardson, & Burke, 2006) and COLONY (Jones & Wang, 2010). MasterBayes was used as the primary parentage assignment program because of its ability to incorporate phenotypic data, which can result in larger numbers of higher confidence assignments. COLONY was used both to confirm the MasterBayes assignments and to assign sibships among individuals with one or both unsampled parents. The latter provides putative information about the relationships among founders and immigrants rather than assuming that they are unrelated.

For the MasterBayes analysis, we specified the following strict requirements for assigning parentage: (i) fathers had to be alive on the estimated date of conception of the focal pup; (ii) mothers had to be alive on the date of birth and present in the pack where the focal pup was born; (iii) both parents had to be at least six months of age during the month of conception of the focal pup; (iv) offspring could not be their own parents. To maximise confidence in parentage assignments, we also incorporated the following phenotypic data: (i) age and age^2, as reproduction increases with age before tailing off later in life (Sanderson et al., 2015); (ii) whether a female was recorded as having given birth within four weeks of the month in which the pup was born; (iii) whether the male was present in the offspring's pack during the month of conception. MasterBayes was run for 9,772,000 iterations with a burn in of 750,000 and a thinning interval of 9,022. In order to keep the Metropolis Hastings acceptance rate between 0.2 and 0.5, the tuning parameters were set to tunePed (beta=0.3, USdam= 0.03, USsire=0.03). Successive samples from the posterior distribution had low autocorrelation ($r < 0.1$). MasterBayes parentage assignments were accepted if they had an associated probability greater than or equal to 0.8, although the average assignment probability was 0.99.

Additionally, COLONY was used to assign individuals to full- and half-sibship groups. Candidate parent and exclusion parent lists for input into COLONY were generated using the same criteria as for MasterBayes. No maternal or paternal sibships were excluded. We specified a sibship prior of 1.5 for both maternal and paternal average sibship size. This was based on prior knowledge of the breeding system and helped to prevent COLONY from incorrectly grouping offspring into large clusters of false siblings. The probability of a true parent being in the candidate list was set to 0.8 and COLONY assignments were only accepted if they had a probability greater than or equal to 0.8. MasterBayes parentage assignments were accepted first and COLONY assignments were then added where MasterBayes failed to confidently assign parentage.

Derivation of pedigree f and multilocus heterozygosity

Based on the final pedigree, which incorporated information on putative relationships among founders as described above, pedigree inbreeding coefficients (f_{ped}) were calculated for all individuals using the R package pedantics (Morrissey, 2014). However, subsequent analyses involving f_{ped} were based only on individuals with all four grandparents assigned. From the microsatellite data, we also quantified each individual's standardised multilocus heterozygosity (sMLH) using inbreedR (Stoffel et al., 2016). The same program was also used to calculate g_2, a quantity that estimates identity disequilibrium (the extent to which heterozygosities are correlated across loci) following David, Pujol, Viard, Castella, & Goudet (2007). We also used inbreedR to calculate the 95% confidence interval of g_2 by bootstrapping over individuals and to permute the genetic data to generate a p-value for the null hypothesis of no variance in inbreeding in the sample (i.e. $g_2 = 0$) as described in detail by Stoffel et al. (2016).

Testing for parentage assignment biases in our pedigree

The majority of accepted parental relationships had very high confidence (89% at ≥ 99% confidence). Nevertheless, Wang (2010) showed that parentage analyses can potentially be biased in favour of heterozygotes, which could potentially create an artefactual positive relationship between sMLH and reproductive success. We evaluated whether such a bias could affect our pedigree by testing for an association between parental heterozygosity and the confidence with which parents were assigned in our pedigree using a generalised linear model (GLM) with a binomial error structure. A slight but statistically significant bias was found in the direction of homozygotes being assigned parentage with slightly greater confidence than heterozygotes (Supplementary Table S1). To explore this further, we simulated pedigrees based on the empirical allele frequencies of our study population. Our methods and results are described in detail in the supplementary information. Briefly, initial simulations assuming random mating assigned 94% of parents with a probability of 1.0 and therefore no bias could be detected. Hence, we simulated an arguably more realistic pedigree with close inbreeding for which parentage analysis should be technically more challenging due to high relatedness among the candidate parents. Consistent with results from our empirical dataset, we found that homozygotes had a slightly higher probability of being assigned parentage (Supplementary Table S2). Taken together, these findings suggest that any bias in our pedigree should be both small and in the opposite direction to that predicted, and is therefore unlikely to generate a false signal of inbreeding depression.

Statistical analyses

Strong inbreeding depression early in life will tend to deplete the adult population of inbred individuals and thereby reduce the power to detect inbreeding effects later in life (Huisman et al., 2016). To evaluate this possibility, we grouped individuals into six cohorts based on their survival to a given age (< one, one, two, three, four or ≥ five years old) and used Levene's test to assess the equality of variances of f_{ped} and sMLH among the cohorts and Spearman's rank to test for a decrease in mean inbreeding with increasing age. We then investigated inbreeding depression for four main fitness components: (i) survival to nutritional independence; (ii) survival beyond nutritional independence; (iii) yearling body mass; and (iv) annual reproductive success (see below for further details). These fitness components were used as response variables in four separate analyses conducted within R version 3.2.3 (R Core Team, 2014). Beforehand, all of the explanatory variables were checked for collinearity using pair plots and by calculating pairwise correlation coefficients. Graham (2003) showed that correlations between explanatory variables as low as 0.28 may compromise model parameterisation but collinearity in our models was well below this, except for f_{ped} and sMLH, which we dealt with as described below. All of our models were also validated though visual inspection of histograms of residuals and plots of residuals against fitted values for each of the explanatory variables as recommend by Zuur, Ieno, & Saveliev (2009).

For each analysis, we constructed a set of competing models, each incorporating prior knowledge of the banded mongoose system, and quantified their relative support using $\mathrm{AIC_c}$-weights within a multi-model inference framework. As support for a model increases, its $\mathrm{AIC_c}$-weight tends towards 1. To quantify the contributions of individual predictor variables, we then calculated predictor-$\mathrm{AIC_c}$-weights by summing the $\mathrm{AIC_c}$-weights of all models containing that predictor. We also followed the recommendation of Richards et al. (2011) and discarded models with better supported models nested within them (i.e. models that are more complicated versions of a better supported model).

Within the above framework, f_{ped} and sMLH were used as predictor variables to quantify the effects of inbreeding on fitness. Including f_{ped} and sMLH in the same models is likely to cause problems due to multi-collinearity because both are estimates of $\mathrm{IBD_g}$. Therefore, we quantified any potential effects of sMLH above and beyond f_{ped} by constructing a set of models containing both f_{ped} and the residuals of sMLH on f_{ped} (henceforth termed residual sMLH). As there is no statistical collinearity between f_{ped} and residual sMLH, we were able to include information from the pedigree and molecular markers simultaneously without biasing the regression parameter estimates (Graham, 2003). Residual sMLH can be interpreted as whether an individual is more or less heterozygous than expected given their f_{ped} and its effect size can be interpreted as its effect additional to that already made through its relationship with f_{ped} as any variance explained by both terms is attributed to f_{ped}. This technique is called sequential regression and performs well across a range of complex functional relationships and collinearity structures (Dormann et al., 2013). Additional non-genetic explanatory variables were analysed based on prior knowledge of the mongoose system as described below.

(i) Survival to nutritional independence

As mortality is highest in banded mongooses prior to nutritional independence around day 90, we first analysed survival to 90 days. A recent study found that offspring of extra group matings, which tend to be more heterozygous, have higher survivorship to 90 days (Nichols, Cant, & Sanderson, 2015), suggesting that there could be a direct link between inbreeding and early survivorship. In the current study, data were available for a total of 489 individuals with all four grandparents assigned. Survival was analysed as a binomial response variable (coded as 1 = survived, 0 = died) within generalized linear mixed models (GLMMs) using lme4 (Bates, Maechler, Bolker, & Walker,

2015) with litter nested within pack as random effects. A total of 19 competing models were constructed (see Table 1), each containing different combinations of predictor variables representing plausible hypotheses to be evaluated within a multi-model inference framework. We included rainfall during the 30 days prior to birth as a predictor variable in all of the models, as this is robustly associated with early life survival (Nichols et al., 2015; Sanderson et al., 2015). As escorting has a highly significant effect on survival to 60 days (Gilchrist, 2004) but is only weakly associated with survival to 90 days (Hodge, 2005), we also included escorting as a continuous variable (see above) in a subset of the models. To further test for an interaction between inbreeding and stress, we constructed a further subset of models containing interactions between rainfall and one of the inbreeding terms (i.e. rain * f_{ped} or rain * sMLH). As explained above, the effect of residual heterozygosity was evaluated by constructing models containing both f_{ped} and residual sMLH.

Table 1. Alternative models of survival to nutritional independence ranked in order of their AIC_c support. See the Materials and Methods section for further details.

Model	Structure	k	logLikelihood	AIC_c	ΔAIC_c	AIC_c-weight
M5	Rain + escorting	5	-271.954	554.033	0.000	0.348
M7	Rain + escorting + sMLH	6	-271.944	556.061	2.029	0.126
M6	Rain + escorting + f_{ped}	6	-271.953	556.081	2.048	0.125
M1	Rain	4	-274.286	556.655	2.623	0.094
M15	Rain * sMLH + escorting	7	-271.866	557.965	3.932	0.049
M11	Rain * f_{ped} + escorting	7	-271.917	558.066	4.034	0.046
M8	Rain + escorting + f_{ped} + residual sMLH	7	-271.939	558.110	4.078	0.045
M3	Rain + sMLH	5	-274.263	558.651	4.618	0.035
M2	Rain + f_{ped}	5	-274.282	558.688	4.655	0.034
M16	Rain * residual sMLH + escorting + f_{ped}	8	-271.811	559.923	5.890	0.018
M12	Rain * f_{ped} + escorting + residual sMLH	8	-271.902	560.103	6.071	0.017
M13	Rain * sMLH	6	-274.182	560.539	6.506	0.013
M9	Rain * f_{ped}	6	-274.203	560.580	6.547	0.013
M4	Rain + f_{ped} + residual sMLH	6	-274.248	560.669	6.637	0.013
M18	Rain * (f_{ped} + residual sMLH) + escorting	9	-271.781	561.937	7.905	0.007
M19	(Intercept only)	3	-278.019	562.087	8.054	0.006
M14	Rain * residual sMLH + f_{ped}	7	-274.091	562.415	8.382	0.005
M10	Rain * f_{ped} + residual sMLH	7	-274.168	562.568	8.536	0.005
M17	Rain * (f_{ped} + residual sMLH)	8	-274.022	564.345	10.312	0.002

(ii) Survival beyond nutritional independence

We investigated inbreeding depression for longevity based on all individuals that survived beyond 90 days (n = 428 mongooses with at least all four grandparents in the pedigree). Lifespan was investigated using Cox-proportional-hazard models in the survival package (Therneau & Grambsch, 2000). Individuals that survived until the end of the study or that emigrated from the study population were classified as right censored in the models. To account for the non-independence of individuals within social groups, we fitted pack as a frailty term, equivalent to a random effect. We also verified that the proportional hazard was independent of time using plots of the scaled Schoenfeld residuals. We constructed 14 competing models (see Table 2), all of which contained sex (coded as female = 0, male =1) because males tend to have a longer lifespan (Cant, Nichols, Thompson, & Vitikainen, 2016). We used mean monthly rainfall in the first year of life as a predictor variable in a subset of models because it is associated with prey abundance and thereby influences lifespan (Marshall et al., 2017). As described above for the models of survival to nutritional independence, we also tested for an interaction between inbreeding and stress by constructing models containing interactions between rainfall and the inbreeding terms.

Table 2. Alternative models of survival beyond nutritional independence ranked in order of their AIC_c support. See the Materials and Methods section for further details.

Model	Structure	k	LogLikelihood	AIC_c	ΔAIC_c	AIC_c-weight
M7	Sex + rain + sMLH	8.5	-1645.576	3297.209	0.000	0.261
M11	Sex + rain * sMLH	9.4	-1644.911	3297.916	0.707	0.183
M1	Sex	6.9	-1647.964	3297.938	0.728	0.181
M3	Sex + sMLH	8.1	-1647.174	3298.376	1.167	0.145
M5	Sex + rain	6.3	-1647.560	3299.149	1.939	0.099
M8	Sex + rain + f_{ped} + residual sMLH	7.9	-1646.837	3301.768	4.559	0.027
M2	Sex + f_{ped}	6.6	-1649.023	3302.074	4.865	0.023
M4	Sex + f_{ped} + residual sMLH	7.8	-1648.015	3302.086	4.876	0.023
M6	Sex + rain + f_{ped}	6.6	-1648.164	3302.385	5.176	0.020
M12	Sex + rain * residual sMLH + f_{ped}	8.6	-1646.418	3302.979	5.769	0.015
M10	Sex + rain * f_{ped} + residual sMLH	9.0	-1646.708	3303.559	6.350	0.011
M9	Sex + rain * f_{ped}	7.7	-1648.083	3304.261	7.052	0.008
M13	Sex + rain * (f_{ped} + residual sMLH)	9.7	-1646.283	3304.765	7.555	0.006
M14	(Intercept only)	4.9	-1650.698	3322.777	25.568	0.000

(iii) Yearling body mass

We next investigated inbreeding depression for body mass (measured in g) at one year of age. Heavier banded mongoose females breed earlier (Hodge, 2005) and may thus have higher lifetime reproductive success. Also, yearling body mass exhibits inbreeding depression (Sanderson et al., 2015) although the study in question did not analyse microsatellite heterozygosity. Individuals were habituated to step onto a portable weighing balance for a small reward of milk, which allowed us to measure body mass. Yearling body mass was calculated as the average of all morning mass measurements for an individual taken between 350 and 380 days of age. Measurements were taken in the morning to standardise against fluctuations in body mass that may occur during the day. Data on yearling body mass were available for a total of 156 individuals with all four grandparents known. We constructed 53 competing models (See Table 3) with litter nested within pack as random effects. These models were run in the glmmADMB package (Fournier, Skaug, Ancheta, & Ianelli, 2012) with a Gaussian error distribution. We included sex in a subset of models and rainfall in the 30 days prior to birth in a subset of the models as this was previously found to be positively associated with body mass in one study (Nichols et al., 2015) but not in another (Sanderson et al. 2015). To test for interactions between inbreeding and stress, some of these models also included interactions between rainfall and the inbreeding terms. Escorting was included in a further subset of models as it correlates positively with pup weight at 84 days (Hodge, 2005; but see Gilchrist, 2004).

Table 3. Alternative models of yearling body mass ranked in order of their AIC_c support. See the Materials and Methods section for further details. Only models with AIC_c-weights greater than 0.01 are shown.

Model	Structure	k	logLikelihood	AIC_c	ΔAIC_c	AIC_c-weight
M28	Sex + f_{ped}	6	-930.982	1874.551	0.000	0.325
M32	Sex + rain + f_{ped}	7	-930.896	1876.581	2.029	0.118
M36	Sex + index + f_{ped}	7	-930.935	1876.659	2.107	0.113
M30	Sex + f_{ped} + residual sMLH	7	-930.955	1876.699	2.147	0.111
M43	Sex + rain * f_{ped}	8	-930.509	1878.039	3.488	0.057
M34	Sex + rain + f_{ped} + residual sMLH	8	-930.849	1878.719	4.168	0.040
M40	Sex + rain + index + f_{ped}	8	-930.849	1878.719	4.168	0.040
M38	Sex + index + f_{ped} + residual sMLH	8	-930.910	1878.841	4.290	0.038
M44	Sex + rain * f_{ped} + residual sMLH	9	-930.436	1880.158	5.606	0.020
M45	Sex + escorting + rain * f_{ped}	9	-930.448	1880.182	5.630	0.019
M48	Sex + rain * residual sMLH + f_{ped}	9	-930.644	1880.574	6.022	0.016
M42	Sex + rain + index + f_{ped} + residual sMLH	9	-930.804	1880.894	6.342	0.014
M27	Sex	5	-935.417	1881.251	6.699	0.011
M2	f_{ped}	5	-935.495	1881.407	6.855	0.011

(iv) Annual reproductive success

Reproductive success is closely linked to fitness but no studies of banded mongooses have previously investigated inbreeding depression for this trait. We therefore used the pedigree to quantify annual reproductive success, expressed as the number of pups assigned to each individual, for all animals over six months of age who survived a given year. Because reproductive opportunities differ between the sexes, with most females breeding regularly while male reproductive success is strongly skewed towards the oldest 3–5 males in a pack (Nichols, Amos, Cant, Bell, & Hodge, 2010), separate models were constructed for each sex. These were based on a total of 240 annual observations of 99 females and 354 annual observations of 129 males. Annual reproductive success was modelled using a negative binomial error distribution with zero-inflation within the R package glmmADMB (Skaug, Fournier, Nielsen, & Magnusson, 2013). To account for multiple observations of individuals and packs, we fitted individual and pack as random effects. We constructed 14 competing models separately for females and males (see Tables 4a and 4b respectively). As reproductive success tends to increase with age before tailing off later in life (Sanderson et al., 2015), we included age and age^2 as predictor variables in all of the models. Average monthly rainfall over the year was also included in a subset of models as a proxy for environmental stress, while inbreeding–stress interactions were investigated through the inclusion of models containing interactions between rainfall and the inbreeding terms.

Table 4. Alternative models of annual reproductive success in (a) females, and (b) males, ranked in order of their AIC$_c$ support. The models of female annual reproductive success which included inbreeding–stress interactions failed to converge and so were omitted. See the Materials and Methods section for further details.

(a)

Model	Structure	k	logLikelihood	AIC$_c$	ΔAIC$_c$	AIC$_c$-weight
M4	Age + age^2 + f_{ped}	8	-329.679	675.981	0.000	0.286
M1	Age + age^2	7	-330.848	676.179	0.197	0.259
M5	Age + age^2 + rain + f_{ped}	9	-329.642	678.067	2.085	0.101
M8	Age + age^2 + f_{ped} + residual sMLH	9	-329.652	678.087	2.105	0.100
M3	Age + age^2 + sMLH	8	-330.790	678.203	2.222	0.094
M2	Age + age^2 + rain	8	-330.808	678.239	2.258	0.092
M7	Age + age^2 + rain + f_{ped} + residual sMLH	10	-329.625	680.211	4.229	0.034
M6	Age + age^2 + rain + sMLH	9	-330.733	680.249	4.267	0.034
M14	(Intercept only)	5	-369.474	749.204	73.223	0.000

(b)

Model	Structure	k	logLikelihood	AIC$_c$	ΔAIC$_c$	AIC$_c$-weight
M8	Age + age^2 + f_{ped} + residual sMLH	9	-300.139	618.801	0.000	0.494
M7	Age + age^2 + rain + f_{ped} + residual sMLH	10	-300.133	620.907	2.106	0.172
M12	Age + age^2 + rain * residual sMLH + f_{ped}	11	-299.697	622.166	3.365	0.092
M10	Age + age^2 + rain * f_{ped} + residual sMLH	11	-300.051	622.874	4.073	0.065
M3	Age + age^2 + sMLH	8	-303.333	623.083	4.282	0.058
M4	Age + age^2 + f_{ped}	8	-303.792	624.001	5.200	0.037
M13	Age + age^2 + rain * (f_{ped} + residual sMLH)	12	-299.663	624.241	5.440	0.033
M6	Age + age^2 + rain + sMLH	9	-303.332	625.187	6.386	0.020
M5	Age + age^2 + rain + f_{ped}	9	-303.779	626.081	7.280	0.013
M11	Age + age^2 + rain * sMLH	10	-302.895	626.431	7.630	0.011
M9	Age + age^2 + rain * f_{ped}	10	-303.725	628.091	9.290	0.005
M1	Age + age^2	7	-309.393	633.110	14.308	0.000
M2	Age + age^2 + rain	8	-309.390	635.197	16.396	0.000
M14	(Intercept only)	5	-343.651	697.474	78.673	0.000

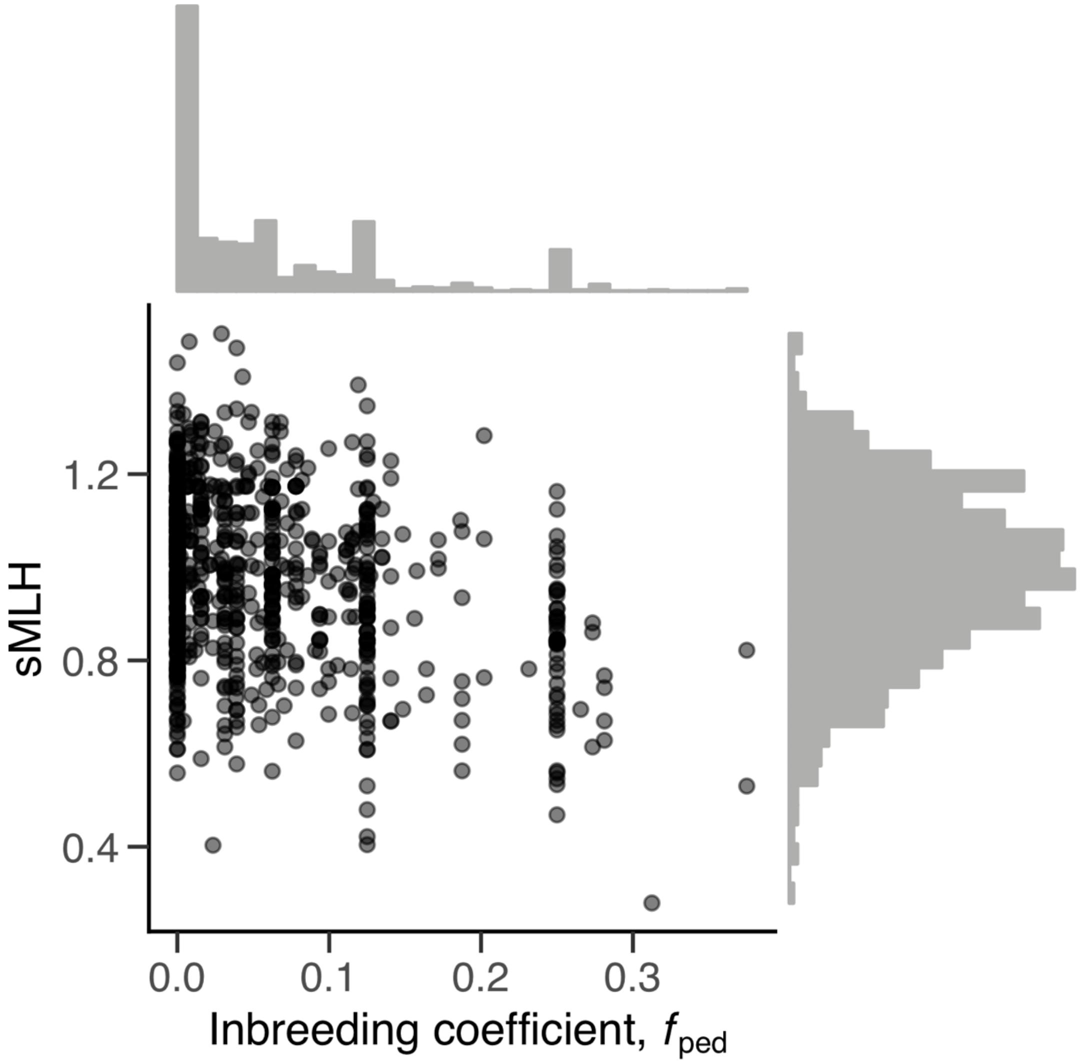
Results

We augmented an existing microsatellite dataset comprising 1,748 individuals genotyped at 35–43 microsatellite loci (Sanderson et al., 2015) by genotyping an additional 192 individuals at 35 microsatellites. This allowed us to enlarge the nine-generation deep banded mongoose pedigree of Sanderson et al. (2015) by increasing the number of maternal links from 1,570 to 1,725 and the number of paternal links from 1,476 to 1,625. The restricted dataset of individuals with all four grandparents assigned, which formed the basis of all subsequent analyses, increased from 672 to 777.

Inbreeding and heterozygosity

Our pedigree uncovered appreciable variance in inbreeding (mean $f_{\text{ped}} = 0.058$, variance = 0.006), with the majority of individuals (66.4%) being to some extent inbred (Figure 1, top marginal histogram). Weak inbreeding ($0 < f_{\text{ped}} < 0.125$) accounted for 46.5% of the population, while 12.9% of individuals were moderately inbred ($0.125 \leq f_{\text{ped}} < 0.25$) and 7.1% were closely inbred ($f_{\text{ped}} \geq 0.25$). Microsatellite heterozygosity (sMLH) was approximately normally distributed with a mean of 0.982 and a variance of 0.034 (Figure 1, right marginal histogram) and correlated significantly with f_{ped} ($R = -0.34$, $p < 0.001$). Furthermore, the measure g_2, which quantifies the extent to which heterozygosity is correlated across loci, was positive (0.012, 95% CI = 0.007–0.018) indicating that the microsatellites are capturing variation in inbreeding. As observed in other species (e.g. Huisman et al. 2016), appreciable variation was observed in sMLH among individuals with the same f_{ped}.

Figure 1. The relationship between the pedigree-based inbreeding coefficient, f_{ped} and sMLH for 777 banded mongoose individuals with all four grandparents assigned (R = 0.34, $p < 0.001$). Scatter on the y-axis for a given f_{ped} value represents variation in microsatellite heterozygosity among individuals with the same pedigree inbreeding coefficient. Marginal histograms show the distributions of f_{ped} (top) and sMLH (right axis).



Changes in inbreeding with age

If inbred individuals experience stronger viability selection early in life, the variance in inbreeding should be lower in adults, making it more difficult to detect inbreeding depression for late-acting traits (Huisman et al., 2016). To investigate this possibility, we divided the mongooses into six cohorts based on their survival to a given age (see Materials and methods) and tested for differences in the variance of f_{ped} and sMLH among these cohorts using Levene's tests. Neither of the inbreeding measures showed a decrease in variance with age (Table S3) and the variance in sMLH did not differ significantly among cohorts ($F_5 = 0.74$, $p = 0.59$). However, the cohorts did not have equal variance in f_{ped} ($F_5 = 2.36$, $p = 0.03$). This result appears to be driven by low sampling variance in individuals who survived between one and two years as the variance in f_{ped} no longer differed significantly among cohorts after these animals were excluded from the analysis. Taken together, these findings suggest that viability selection against inbred individuals does not reduce the variance in inbreeding with age. In line with this, we also found no evidence for a decline in the mean level of inbreeding with increasing age (f_{ped} *rho* = 0.043, $p = 0.23$; sMLH *rho* = -0.01, $p = 0.78$; Table S3).

Survival to nutritional independence

We found that the model of survival to nutritional independence with the greatest AIC_c support included rainfall in the 30 days prior to birth and escorting as fixed effect explanatory variables (Table 1, intercept = -0.54 ± 0.45 SE, rainfall ß = 0.36 ± 0.13 SE, escorting ß = 0.88 ± 0.41 SE, random effects: pack SD = 0.000, litter nested within pack SD = 1.57). The second and third most supported models included rain and escorting as well as an inbreeding term (Table 1). However, as they had the best model nested within them (i.e. they were more complex but less supported versions of the first model) we did not consider them further, as recommended by Richards et al. (2011).

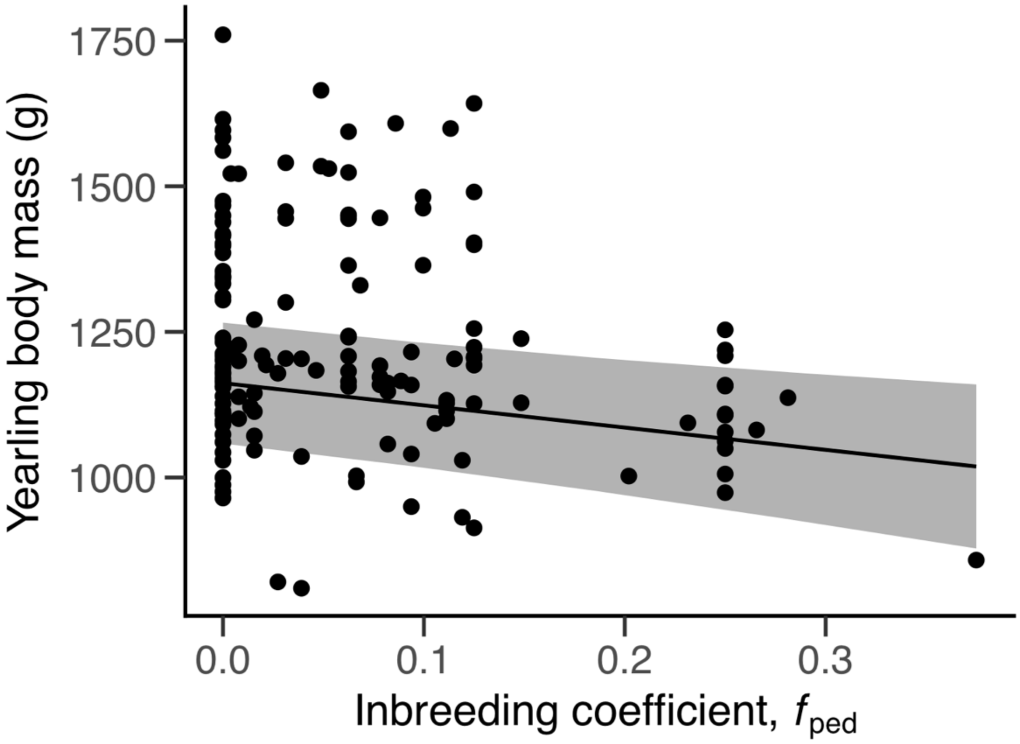
Survival beyond nutritional independence

The results of our analysis of adult survival were equivocal (Table 2). The highest ranking model included sMLH but had roughly equivalent AIC_c support ($\Delta\text{AIC}_\text{c} < 1$) to a simple model that included only sex. As AIC_c tends to slightly favour complex models, especially when there is uncertainty over the best model (Symonds & Moussalli, 2011), our results do not provide convincing evidence of inbreeding depression for longevity.

Yearling body mass

By contrast, strong support was found for inbreeding depression in yearling body mass, with all of the top 12 models containing f_{ped} as a fixed effect explanatory variable (Table 3) and the predictor-AIC$_c$-weight for f_{ped} being high at 0.96. The top ranking model contained sex and f_{ped} (Table 3, Figure 2; intercept = 1162 ± 53 SE, sex ß = 59 ± 19 SE f_{ped} ß = -382 ± 127 SE, random effects: pack SD = 125.5, litter nested within pack SD = 37.6). As before, we disregarded less supported models with this model nested within them as suggested by Richards et al. (2011).

Figure 2. The relationship between f_{ped} and yearling body mass. The trend line shows the expected body mass of a female yearling and the shaded region shows the 95% confidence interval.

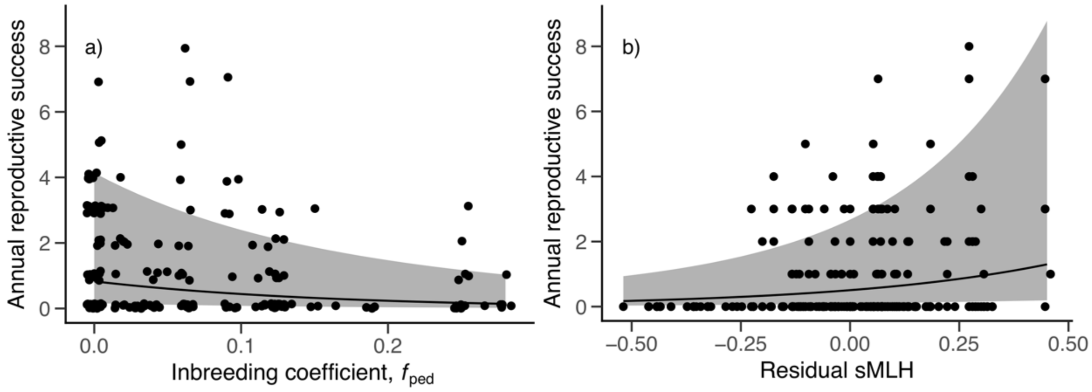


Annual reproductive success

Focusing first on female reproductive success, the top ranking model contained age + age^2 + f_{ped} but the next best model had very similar AIC$_c$ support but did not contain f_{ped} (Table 4a). Because AIC$_c$ support for these two models was so similar and AIC exhibits a slight preference for overly complex models, the simpler model should be preferred. Consequently, our data provided only limited support for inbreeding depression for female annual reproductive success as our preferred model contained only age and age^2 (intercept = -1.25 ± 0.38 SE, age ß = 0.76 ± 0.18 SE, age^2 ß = -0.05 ± 0.02 SE). By contrast, the best supported model for males contained both f_{ped} and residual sMLH (intercept = -2.95 ± 0.48 SE, age ß = 1.45 ± 0.19 SE, age^2 ß = -0.13 ± 0.02 SE, f_{ped} ß = -6.30 ± 1.72 SE, residual sMLH ß = 2.09 ± 0.76 SE). This not only provides evidence for inbreeding depression for male annual reproductive success, but also suggests that marker heterozygosity captures a significant amount of variance that is not explained by f_{ped}. This model was nested within the second and third highest ranking models, which also had considerable AIC$_c$ support and respectively contained rain and an interaction between rain and f_{ped}.

Consistent with theoretical expectations, the best supported model of annual male reproductive success revealed a negative association with f_{ped} (Figure 3a) and a positive association with residual sMLH (Figure 3b). Inbred males with an f_{ped} value of 0.25 were predicted by the model to have approximately 79% fewer offspring than fully outbred individuals with an f_{ped} value of zero, while males with residual sMLH values one standard deviation above zero (0.185) were predicted to have 47% more offspring than individuals with residual sMLH equal to zero. This indicates that within f_{ped} classes, relatively heterozygous individuals tend to have greater reproductive fitness.

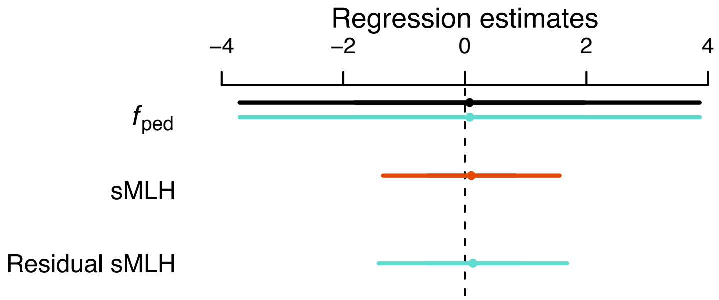
Figure 3. The relationship between annual male reproductive success and a) f_{ped}, and b) residual sMLH derived from a single model (M8 in Table 4b) where both inbreeding measures are fitted together. The trend line shows expected values based on average age and the shaded region shows associated 95% confidence intervals. Data points in plot a) were given a small amount of jitter to avoid over plotting.



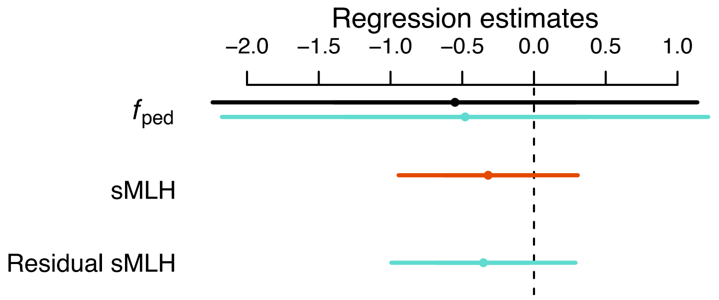
Effect sizes of the inbreeding terms

To provide further insights into the effect sizes of the inbreeding terms, we constructed three alternative models separately for each fitness trait. These models contained non-inbreeding terms that were retained in the top ranking models described above for each trait, while in addition the first model contained f_{ped}, the second contained sMLH and the third contained f_{ped} plus residual sMLH. To evaluate inbreeding effects, we then calculated effect sizes and their corresponding 95% confidence intervals (CIs) for all of the predictor variables contained in each model. The results are summarised separately for each trait in Figure 4. Consistent with results from the information theoretic approach, the 95% CIs of the effect sizes of all three inbreeding terms overlapped zero for survival to nutritional independence, survival beyond nutritional independence and female reproductive success (Figure 4a, b and d), suggesting that there is very little evidence for inbreeding depression for these traits. Also as expected, f_{ped} had negative point estimates whose corresponding 95% CIs did not overlap zero in models of yearling body mass and annual male reproductive success (Figure 4c and e), while sMLH and residual sMLH only had positive estimates and 95% CIs not overlapping zero in models of male reproductive success (Figure 4e).

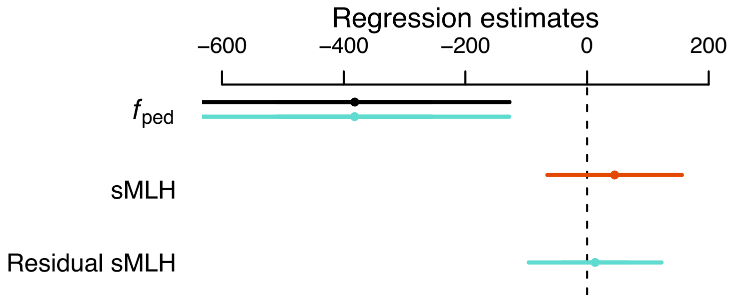
a) Survival to nutritional independence



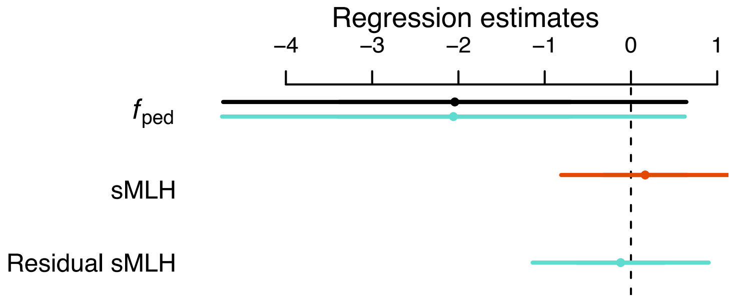
b) Survival beyond nutritional independence



c) Yearling body mass



d) Female annual reproductive success



e) Male annual reproductive success

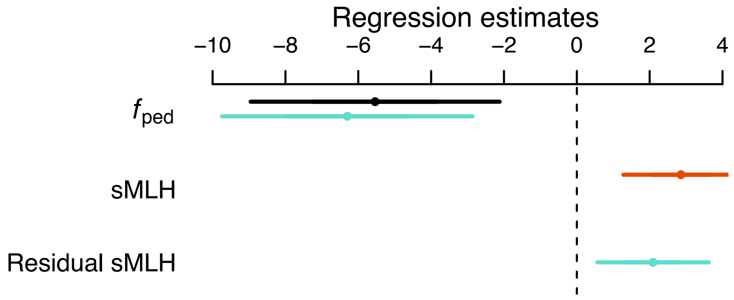


Figure 4. Estimated regression coefficients of the three inbreeding terms in models of five different fitness traits, showing point estimates and associated 95% confidence intervals. Each panel shows three different models–one containing f_{ped} (shown in black), one containing sMLH (shown in dark orange) and one containing f_{ped} + residual sMLH (shown in light turquoise) as described in the Results section. In addition to these inbreeding terms, all of the models contained other fixed effects but these are not shown for ease of interpretation. The larger confidence intervals of f_{ped} relative to sMLH result from its smaller range (Figure 1).

Associated p- and R^2 values

In order to evaluate the sensitivity of our results to the statistical framework employed, we determined the statistical significance of f_{ped}, sMLH and residual sMLH using a frequentist approach. Separately for each trait, we derived *p*-values for each of the inbreeding terms using likelihood ratio tests. The significance of f_{ped} and sMLH was derived by comparing models containing these terms with equivalent 'null models' containing only the relevant non-inbreeding terms, while *p*-values for residual sMLH were obtained through the comparison of models containing f_{ped} plus residual sMLH with equivalent models containing only f_{ped}. To provide an indication of the proportion of variance explained by each model, we also calculated conditional R^2 values for GLMMs (Nakagawa & Schielzeth, 2013) and Cox and Snell's pseudo R^2 values for Cox proportional hazard models (Cox & Snell, 1989). However, this was not possible for zero-inflated negative binomial GLMMs so we instead report log likelihood values for these models (Table 5). To allow direct comparison with other studies, correlation coefficients between the two inbreeding measures and each fitness trait are also provided in the supporting information (Table S4). Consistent with the results of the multi-model approach described above, we found a highly significant effect of f_{ped} on yearling body mass, which explained almost 5% of the total variation (Table 5c), although sMLH did not explain a significant amount of variance in this trait. By contrast, both f_{ped} and sMLH explained significant variation in male annual reproductive success (Table 5e). Furthermore, adding residual sMLH to a model containing only f_{ped} resulted in a significant improvement to the model of annual male reproductive success ($p = 0.007$, Table 5e), suggesting that for some traits genetic markers may capture variation in inbreeding above and beyond that explained by f_{ped}.

Table 5. Statistical significance and variance explained by inbreeding terms in models of five fitness traits. The significance of f_{ped} and sMLH was derived by comparing models containing these terms with equivalent 'null models' containing only the relevant non-inbreeding terms, while *p*-values for residual sMLH were obtained through the comparison of models containing f_{ped} + residual sMLH with equivalent models containing only f_{ped}. For each trait, the models that we constructed are listed in the first column of the table, with the null model shown first. Conditional R^2_{glmm} was calculated following Nakagawa & Schielzeth (2013) and Cox and Snells's pseudo R^2 was calculated using the number of uncensored observations rather than the total number of observations as recommended by O'Quigley et al. (2005). As R^2 values cannot be calculated for zero-inflated negative binomial GLMMs, log likelihood values are presented as a measure of the fit of models of annual male reproductive success.

a) Survival to nutritional independence

Binomial GLMM, $n = 489$

Structure	Likelihood ratio	*p*-value	Conditional R^2_{glmm}
Rain + escorting			0.4701
Rain + escorting + f_{ped}	0.0017	0.9671	0.4702
Rain + escorting + sMLH	0.0213	0.8839	0.4703

b) Survival beyond nutritional independence

Cox proportional hazard model, $n = 428$

Structure	Likelihood ratio	*p*-value	Cox and Snell's pseudo R^2
Sex			0.0817
Sex + f_{ped}	2.1178	0.1456	0.0755
Sex + sMLH	1.5803	0.2087	0.0863

c) Yearling body mass

Gaussian GLMM, $n = 150$

Structure	Likelihood ratio	*p*-value	Conditional R^2_{glmm}
Sex			0.5734
Sex + f_{ped}	8.87	0.0029	0.6221
Sex + sMLH	0.674	0.4117	0.5766

d) Female annual reproductive success

Zero-inflated, negative binomial GLMM, $n = 240$

Structure	Likelihood ratio	*p*-value	Log Likelihood
Age + age^2			-330.848
Age + age^2 + f_{ped}	2.338	0.1263	-329.679
Age + age^2 + sMLH	0.116	0.7334	-330.790

e) Male annual reproductive success

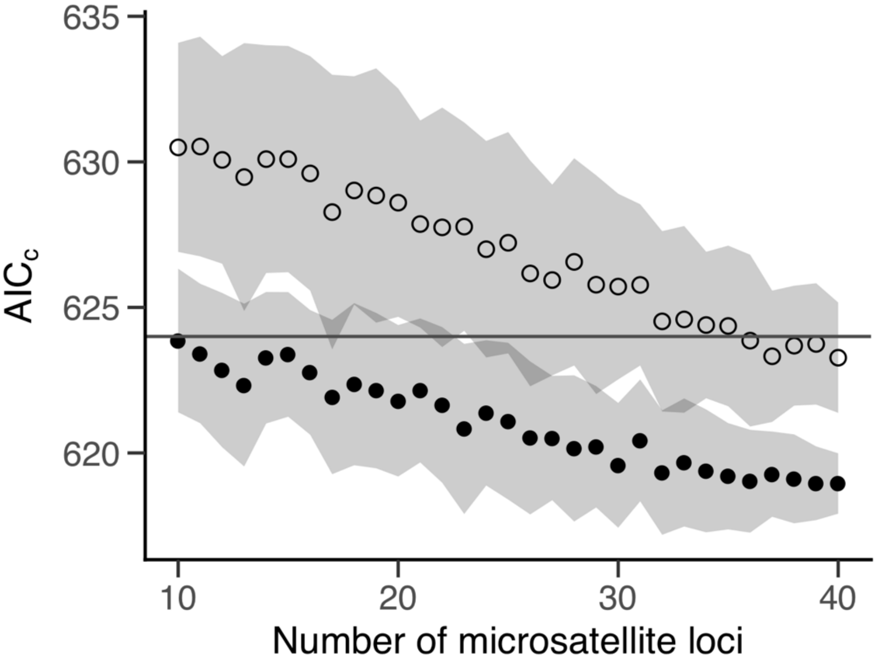
Zero-inflated, negative binomial GLMM, $n = 354$

Structure	Likelihood ratio	*p*-value	Log Likelihood
Age + age^2			-309.393
Age + age^2 + f_{ped}	11.202	0.0008	-303.792
Age + age^2 + sMLH	12.12	0.0005	-303.333
Age + age^2 + f_{ped} + residual sMLH	7.306	0.0069	-300.139

Sensitivity to marker number

To further investigate the explanatory power of f_{ped} and marker heterozygosity, we directly compared three of our models of annual male reproductive success in which the inbreeding terms were f_{ped} (M4 in Table 4b), sMLH (M3 in Table 4b) and f_{ped} plus residual sMLH (M8 in Table 4b) respectively, and explored the sensitivity of model AIC_c to marker number. As expected, AIC_c decreased steadily with increasing marker number (Figure 5). With fewer than around 20 markers, sMLH did not perform as well as f_{ped}, but with 30–40 markers AIC_c values for the two models were very similar. Furthermore, the model containing both f_{ped} and residual sMLH became increasingly superior to the model containing only f_{ped} as more markers were deployed.

Figure 5. The relationship between AIC_c of models of annual male reproductive success and the number of microsatellites used to calculate standardised multilocus heterozygosity. Open points represent models with the structure: age + age^2 + sMLH; closed points represent models with the structure: age + age^2 + f_{ped} + residual sMLH. The horizontal line represents a model with the structure: age + age^2 + f_{ped}. We selected n different microsatellite loci at random and calculated heterozygosity as sMLH 100 times for each value of n. Points represent mean values and the shaded regions indicate ± 1sd.



Testing for local effects

Finally, we tested for the possible involvement of local effects involving specific microsatellite loci by adapting the approach of Szulkin, Bierne, & David (2010). Specifically, we compared a model of male reproductive success containing age, age^2, f_{ped}, and residual sMLH with a model in which residual sMLH was replaced by separate terms for the residual heterozygosity of each of the microsatellite loci. The second model was not a significant improvement over the first, although the corresponding *p*-value was close to significance ($-2\mathrm{LL}_{30} = 42.06$, $p = 0.07$). Our results are therefore more consistent with inbreeding depression than with a mechanism based on one or a small number of local effects.

Discussion

Although inbreeding depression is known to be important in many wild populations, relatively few studies are large and detailed enough either to compare multiple traits at different stages in the life cycle or to investigate the relative explanatory power of pedigree-based and molecular estimates of inbreeding. We therefore used an exceptionally comprehensive long-term study of banded mongooses both to quantify inbreeding depression for early and late-acting traits and to evaluate the hypothesis that marker heterozygosity may capture fitness variation above and beyond that explained by f_{ped}. Contrary to our initial expectations, we did not find evidence for strong viability selection against inbred individuals early in life, but instead detected inbreeding depression for traits relating to individual quality (i.e. yearling body mass and male annual reproductive success). Furthermore, we found that fitting f_{ped} and residual sMLH together in a single model explained significantly more of the variance in male annual reproductive success than using f_{ped} alone. However, this was not the case for yearling body mass, where f_{ped} explained variation in fitness but sMLH did not.

Inbreeding depression for different traits

Theory predicts that inbreeding depression should be greatest for traits closely linked to fitness because traits under strong directional selection will exhibit greater directional dominance (Lynch & Walsh, 1998). This is supported by a meta-analysis that found stronger inbreeding depression for life history traits such as survival and fecundity than for morphological traits such as body weight (DeRose & Roff, 1999). Given that all of the traits we analysed in banded mongooses are arguably very closely linked to fitness, we were initially surprised not to find inbreeding depression for either survival to nutritional independence or longevity. One potential explanation for this is that inbreeding depression for early survival could be buffered by the social system of this species (Ihle, Hutter, & Tschirren, 2017; Nielsen et al., 2012; Pilakouta, Jamieson, Moorad, & Smiseth, 2015) especially if escorts preferentially direct care towards inbred individuals (Thünken, Bakker, Baldauf, & Kullmann, 2007). However, due to the complexity of the banded mongoose system, testing this hypothesis lies beyond the scope of the current study. Alternatively, as the environment is relatively benign and major causes of death in our study population are predation and injuries sustained during aggressive interactions between social groups (Cant et al., 2013), there may be relatively little scope for strong genetic effects on survival. A further possibility is that our study may have lacked the statistical power to detect inbreeding depression for traits with smaller available sample sizes, such as female annual reproductive success. We could have slightly increased our sample size by including the offspring of residents and immigrants to the study site and assuming they were outbred ($f_{\text{ped}} \approx 0$). Currently these individuals were omitted because they do not have all four grandparents confidently assigned. However, as these individuals would all be treated as outbred it is unlikely that they would add much statistical power to detect inbreeding depression. Low sample size seems unlikely to account for the absence of detectable inbreeding depression for early-acting traits like survival to nutritional independence as sample sizes for these analyses were more than double what was available for yearling body mass, where inbreeding depression was detected. Nevertheless, we cannot discount the possibility that inbreeding depression might influence survival at an even earlier stage of development, for instance *in utero* or during their first month *post partum* before emergence from the underground den.

As several studies have shown that inbreeding depression can be magnified by stress (Armbruster & Reed, 2005; Fox & Reed, 2011; Meagher, Penn, & Potts, 2000; Norén, Godoy, Dalén, Meijer, & Angerbjörn, 2016; Reed, Fox, Enders, & Kristensen, 2012), we included interactions between rainfall and both measures of inbreeding in all of our analyses as rainfall is a proxy for food availability. We found that none of the top ranking models of survival to nutritional independence, longevity, yearling body mass or annual reproductive success contained interactions between rainfall and either f_{ped} or sMLH. Furthermore, although rainfall has a strong effect on survival to nutritional independence (Nichols et al., 2015; Sanderson et al., 2015) and was therefore included as a main effect in all models of this particular trait, rainfall did not feature in any of the chosen models of the other three fitness traits. Thus, our rainfall measures do not appear to strongly influence most of the investigated traits, which may help to explain why interactions involving rainfall were not found.

Alternatively, social stressors might be disproportionately important in this cooperative breeding species. Consistent with this, strong inbreeding depression was found for male annual reproductive success, with closely inbred individuals ($f_{\mathrm{ped}} \geq 0.25$) having 79% lower annual reproductive success than individuals with an f_{ped} of zero, whereas our results for female reproductive success provided at best limited support for inbreeding depression. Although the sample size of female observations was smaller, sex-specific inbreeding depression would be consistent with previous studies of wild mice showing that male-male competition amplifies inbreeding depression (Meagher et al., 2000). It would also be in line with stronger reproductive skew in male versus female banded mongooses (Nichols et al., 2010) as stronger directional selection is expected to increase inbreeding depression.

Detecting inbreeding depression with pedigrees and genetic markers

Pedigrees have for many years been the gold standard for quantifying inbreeding depression in wild populations (Pemberton, 2004, 2008). However, pedigree data are often incomplete and assignment errors can introduce significant error into the estimation of f_{ped} (Reid et al., 2014) while the assumption that the founders are outbred and unrelated to one another may also be violated in closed or structured populations. In addition, f_{ped} is a measure of the expected IBD_g of an individual based on its pedigree and cannot capture stochastic variation in realised IBD_g resulting from Mendelian segregation (Hedrick et al., 2016; Hill & Weir, 2011; Knief et al., 2017). Consequently, there has been growing interest in the extent to which f_{ped} and marker heterozygosity can capture inbreeding effects, either independently or when analysed together, as well as in how the explanatory power of genetic markers varies with the number of loci that can be genotyped.

Several studies have compared the ability of pedigrees and microsatellites to detect inbreeding depression. These have reached the general consensus that f_{ped} usually performs better (e.g. Ólafsdóttir & Kristjánsson, 2008; Slate et al., 2004; Taylor et al., 2010), even when hundreds of microsatellites are used (Nietlisbach et al., 2017), although it is also to be expected that tens of thousands of SNPs will outperform f_{ped} (Huisman et al., 2016; Kardos et al., 2015). Nevertheless, both Forstmeier et al. (2012) and Hammerly et al. (2013) detected stronger inbreeding effects with around ten microsatellites than with f_{ped}. Our results fall somewhere in between these opposite ends of the spectrum, with heterozygosity based on around 40 microsatellites having roughly equivalent explanatory power to f_{ped} for male annual reproductive success but not for yearling body mass. This probably reflects a variety of factors as discussed below.

First, most pedigrees suffer to a greater or lesser extent from errors in the assignment of parental relationships, which can lead to significant and often downward bias in the estimation of inbreeding depression (Reid et al., 2014). This could partly explain the contrasting results of Nietlisbach et al. (2017) and Hammerly et al. (2013), as the former study was able to genotype the parents of all of the individuals used in the analysis for a very large number of microsatellites, resulting in an unusually accurate pedigree, whereas Hammerly et al. (2013) recognised that their pedigree contained a significant number of errors. Although it is difficult to directly compare different studies, our banded mongoose pedigree probably sits closer to the song sparrow end of the continuum, as our panel of microsatellites was moderately large and the majority of the adult population (all but four parents, Sanderson et al. 2015) was included.

A second factor that may influence the relative explanatory power of pedigrees and genetic markers is pedigree depth. Pedigree-based inbreeding estimates become increasingly accurate with increasing depth, although these estimates become only marginally more precise beyond five generations in populations with certain structures (Slate et al., 2004, Kardos et al., 2015). Therefore, deeper pedigrees will tend to capture more of the variance in IBD_{g} within a given population and leave less “undetected inbreeding” for the markers to capture (Nietlisbach et al., 2017). This could potentially help to explain why residual heterozygosity accounts for additional fitness variation in one of the two traits that showed inbreeding depression in our study, as 54% of individuals in the song sparrow pedigree had eight or more known ancestral generations, whereas our equivalent value was only 3% and around half of all individuals in our banded mongoose pedigree had fewer than five generations known. We may also see variation within studies. Residual sMLH may be less informative for individuals with deeper pedigrees as they have less 'undetected inbreeding'. This variation may inflate confidence intervals for the residual sMLH regression coefficient as the true value would differ between individuals. This does not seem to be a problem for our analysis as Figure 4 does not show inflated confidence intervals for residual sMLH relative to sMLH.

Third, the information content of the genetic markers used in a study will influence how well heterozygosity measures inbreeding. Homozygosity measured at genetic markers with few alleles and/or highly skewed allele frequencies is more likely by chance to reflect IBS than IBD and so may provide relatively little information about an individual's level of inbreeding. Calculating the IBD–IBS discrepancy for our dataset following Knief et al. (2017) resulted in an estimate of 49%. This is higher than in zebra finches (13%, Knief et al., 2017) and may in part reflect the relatively low allelic richness of our microsatellites (average number of alleles = 5.2, Supplementary Table S5). However, this does not appear to have been a major issue for our study, probably due to the relatively large panel of available microsatellites. It might be interesting to explore this further in future studies by attempting to develop 'ideal markers' where there is little to no IBD–IBS discrepancy. One possible strategy would be to genotype small panels of SNPs residing within known runs of homozygosity (ROH) following the suggestion of Knief et al. (2017).

In addition, factors intrinsic to a given system may also play a role, such as the frequency of close inbreeding, the number of chromosomes and genetic map length. For example, theoretical work by Hill & Weir (2011) and simulations by Hedrick et al. (2016) suggest that the variation in realised $\mathrm{IBD_g}$ around that expected by f_{ped} will be greater for closer inbreeding, and hence that the type and variance of inbreeding in a population will affect how well f_{ped} estimates $\mathrm{IBD_g}$. We know that close inbreeding is relatively common in banded mongooses, not because of small population sizes but because both sexes frequently remain in their natal group for their entire lives and breed with other group members (Nichols et al., 2014). Hence, the relatively high frequency of close inbreeding in this species could potentially help to explain our results.

Furthermore, f_{ped} will be relatively imprecise in species with fewer chromosomes and shorter genetic maps because genomes inherited in larger blocks will exhibit greater variance in realised $\mathrm{IBD_g}$ for a given value of f_{ped} (Franklin, 1977; Hill & Weir, 2011; Kardos et al., 2015; Stam, 1980). Genomes inherited in larger blocks should therefore provide greater scope to detect inbreeding depression with relatively few molecular markers (Forstmeier et al., 2012). The size of these blocks is partly determined by the number of chromosomes because the proportion of unlinked loci will increase with chromosome number (Weir, Avery, & Hill, 1980), while within chromosomes both the number and distribution of crossovers will play a role (Knief et al., 2017). To illustrate this point, nearly a third of the zebra finches genome segregates in only four blocks because almost half of the autosomal genome comprises four chromosomes that experience very little recombination (Forstmeier et al., 2012). It is currently difficult for us to judge how these factors could have influenced our results as the number of chromosomes in banded mongoose is neither small nor large (2n = 36, Fredga, 1972) and the recombination landscape of this species has not yet been characterised.

Factors that influence the relative ability of f_{ped} and markers to detect inbreeding depression will also vary among populations and are expected to differ systematically between large populations and smaller, threatened ones. Small or fragmented populations often have higher rates of inbreeding and lower genetic diversity and Grueber, Wallis, & Jamieson (2008) argue that these and other differences make it difficult to generalise results from outbred populations to threatened ones. It is therefore worth considering how similar systems are in the prevalence of inbreeding before extrapolating results between them. Furthermore, historical changes in the structure of a population, including bottlenecks and population admixture, may also create variance in inbreeding *sensu lato* (Bierne, Tsitrone, & David, 2000; Grueber et al., 2008; Weir et al., 1980). Consequently, the number of markers needed to accurately quantify IBD_{g} will also depend on the demographic history of the population in question (Miller et al., 2014).

Capturing inbreeding depression with sequential regression

Although pedigrees clearly fail to capture variation in heterozygosity about the genome-wide expectation given by f_{ped}, relatively few studies have attempted to quantify the amount of fitness variation that genetic markers might capture additional to that explained by f_{ped}. Some studies approached this question by fitting f_{ped} and heterozygosity as predictor variables in the same statistical models of the focal traits (e.g. Bensch et al., 2006; Grueber et al., 2011, Nietlisbach et al. 2017). However, this approach may be problematic because heterozygosity is often correlated with f_{ped} and including collinear variables in a model can lead to inaccurate parameter estimates (Graham, 2003). We therefore used sequential regression as an alternative approach that attributes all of the shared variance to f_{ped} and is therefore able to estimate how well marker heterozygosity explains variation in fitness after controlling for f_{ped} without biasing parameter estimates. Using an information theoretic approach, we found that the best model of male annual reproductive success contained residual sMLH as well as f_{ped}. This was also supported by a frequentist approach, which uncovered a highly significant ($p = 0.007$) effect of residual sMLH. By contrast, residual sMLH did not explain significant variation in yearling weight. One potential explanation for this could be that male reproductive success exhibits stronger inbreeding depression, which may make residual heterozygosity effects easier to detect.

An alternative to controlling statistically for f_{ped} is to control for this experimentally by screening genetic markers in individuals chosen to have the same f_{ped}. For example, Hemmings et al. (2012) used 384 genome-wide distributed SNPs to estimate homozygosity in zebra finches with the same f_{ped}, finding that the most homozygous birds were less likely to survive to sexual maturity. This study echoes an earlier paper where full-sibling reed warblers were compared (Hansson, Bensch, Hasselquist, & Åkesson, 2001) and where again heterozygosity correlated with fitness despite identical f_{ped}. A key difference is that Hansson et al. (2001) used five microsatellites, leading the authors to conclude that a local effect was responsible, whereas the much larger panel used by Hemmings et al. (2012) more or less precludes a dominant role for only one or two loci. Consistent with the latter study, two lines of evidence are suggestive of a genome-wide mechanism in banded mongooses. First, in our models of annual male reproductive success, we found that $\mathrm{AIC_c}$ steadily fell as the number of randomly sampled microsatellite loci increased, regardless of whether sMLH or residual sMLH were fitted as predictor variables. Second, we did not find that a model incorporating the single-locus heterozygosities of all of the loci explained significantly more variation than a model containing only sMLH. Although the second test is admittedly conservative, collectively our results point towards a polygenic architecture, consistent with the widespread view that the majority of inbreeding effects are caused by many loci with small effect sizes distributed across the genome (Charlesworth & Willis, 2009; Szulkin et al., 2010).

Future perspectives

Looking to the future, although ours and many other studies have quantified heterozygosity using microsatellites, simulations clearly indicate that tens of thousands of markers will outperform even very deep pedigrees at capturing inbreeding depression, particularly when they can be mapped to a reference genome to quantify ROH (Kardos et al., 2015; Wang, 2016). This is supported by a growing number of empirical studies of wild populations using approaches like restriction site associated DNA sequencing (Hoffman et al., 2014), high density SNP arrays (Chen et al., 2016; Huisman et al., 2016) and whole-genome resequencing (Kardos et al., 2018). As the costs of these and related methods continue to fall, they are likely to become preferred approaches for studying inbreeding and its consequences in wild populations.

Conclusion

We used a high-quality pedigree together with data from up to 43 microsatellites to investigate inbreeding depression in a cooperatively breeding species where mating between close relatives is common. We detected inbreeding depression for yearling body weight and annual male reproductive success but found no evidence for inbreeding affecting survival, either to nutritional independence or beyond. Furthermore, for one out of the two traits exhibiting inbreeding depression, our panel of microsatellites had similar explanatory power to f_{ped} and residual sMLH explained a significant proportion of fitness variation when fitted in a model together with f_{ped}. Our findings therefore suggest that, at least under some circumstances, combining pedigree and molecular measures of inbreeding may allow us to explain more fitness variation and thereby improve our understanding of the genetic variance underpinning fitness variation in wild populations.

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Data Accessibility

Microsatellite genotypes, pedigree inbreeding coefficients, and lifetime and annual data records are available via Dryad doi:10.5061/dryad.bq868sh. All of the computer code used to analyse the data are provided as R script files.

Supporting information

Evaluating potential biases in parentage assignment

We performed three analyses to evaluate whether our parentage assignments could have been biased towards heterozygous individuals.

Analysis of the empirical dataset

First, we tested for an association between parental heterozygosity and the confidence with which parents were assigned in our empirical pedigree. Confidence was modelled as a binomial response variable in a generalised linear model (GLM). A binomial error structure was used because MasterBayes defines confidence as the proportion of times a particular parent is assigned to an offspring in the MCMC chain. The two predictor variables were paternal and maternal sMLH respectively. This model was significantly better than an intercept-only model as indicated by a likelihood ratio test ($-2\mathrm{LL}_{2 = 3030.2}$, $p < 0.0001$). Furthermore, a slight bias was found against heterozygotes (Table S1) such that parents with sMLH values two standard deviations below the mean were predicted to have an assignment probability 0.02 greater than parents with sMLH two standard deviations above the mean.

Table S1. Results of a binomial GLM of the confidence with which parents were assigned in our empirical pedigree. Statistical significance was determined using likelihood ratio tests.

Term	Estimate	Standard Error	$-2LL_1$	p-value
(Intercept)	0.94	0.009		
sMLH sire	-0.13	0.008	265.91	<0.0001
sMLH dam	-0.31	0.007	2232.5	<0.0001

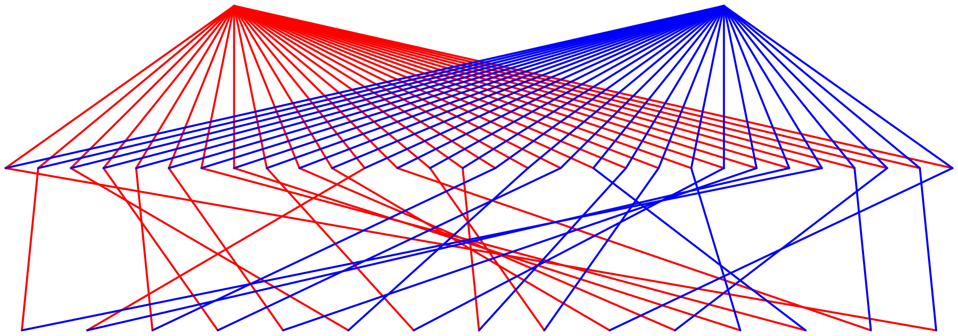
Analysis of a simple simulated pedigree

Second, we simulated random mating between 15 males and 15 females to produce 15 offspring. All genotypes were simulated based on the empirical allele frequencies. The offspring were then assigned parents from among the 30 candidate parents using the R package MasterBayes as described in the Materials and methods section of the manuscript. The above steps were repeated 1000 times. We found that 94% of all simulated offspring were assigned parents with a probability of 1.0 and hence no bias could be detected.

Analysis of a simulated pedigree with close inbreeding

Finally, we simulated a pedigree with close inbreeding, in which parentage assignment is technically more challenging because candidate parents are related and have reduced allelic diversity compared to the total population. We simulated the genotypes of 30 full siblings with a 50:50 sex ratio and then simulated random mating among these individuals to produce 15 inbred offspring. An example pedigree is shown below in Figure S1. We then used MasterBayes to assign parentage to these offspring using the simulated parental generation as candidate parents. This procedure was repeated 1000 times.

Figure S1. An example of a simulated pedigree with close inbreeding. Dam lines are shown in red and sire lines are shown in blue.



As above, we modelled the confidence of parentage assignment using a GLM with a binomial error structure and paternal and maternal sMLH fitted as predictor variables. To assess the significance of this model, we used a likelihood ratio test to compare it with an intercept-only model. As found in our previous analysis of the empirical pedigree, the more complex model explained significantly more variation ($-2LL_2 = 37646$, $p < 0.001$) and a small bias was found against heterozygotes. Specifically, parents with sMLH values two standard deviations below the mean were predicted to be assigned parentage with a probability of 0.038 greater than equivalent individuals with sMLH values two standard deviations above the mean.

Table S2. Results of a binomial GLM of the confidence with which parents were assigned in a simulated pedigree with close inbreeding. Statistical significance was determined using likelihood ratio tests.

Term	Estimate	Standard Error	$-2LL_1$	p-value
(Intercept)	5.14	0.013		
Paternal sMLH	-1.06	0.008	16866	<0.0001
Maternal sMLH	-1.19	0.008	21584	<0.0001

Changes in inbreeding with age

Table S3. Table reporting the mean and variance in f_{ped} and sMLH of individuals split into cohorts based on their survival to at least a given age.

Cohort	Mean f_{ped}	Variance f_{ped}	Mean sMLH	Variance sMLH
0	0.056	0.005	0.984	0.032
1	0.051	0.004	0.992	0.033
2	0.057	0.006	0.954	0.038
3	0.083	0.008	0.967	0.042
4	0.086	0.008	0.996	0.026
5	0.059	0.006	0.986	0.045

Correlation between inbreeding and fitness

To help comparison between studies, the correlation coefficients of f_{ped} and sMLH with each measure of fitness directly are presented in Table S4.

Table S4. Table reporting the correlation coefficient between each of the two measures of inbreeding and all five fitness measures.

Fitness trait	Correlation coefficient of fitness trait with f_{ped}	Correlation coefficient of fitness trait with sMLH
Juvenile survival	-0.00	-0.01
Adult survival	0.09	0.03
Yearling weight	-0.24	0.10
Annual reproductive success ♀	-0.15	0.03
Annual reproductive success ♂	-0.18	0.27

Allele numbers for microsatellites

Table S5. Numbers of alleles found at 43 banded mongoose microsatellites.

Marker	Number of alleles
Mon16	6
Mon17	4
Mon25	8
Mon41	4
Mon69	8
Mon19	7
Mon32	4
Mon38	5
Mon65	3
Mon66	2
Mon67	4
Mon68	5
Mon70	5
Mon29	3
Mon31	6
Mon35	5
Mon36	5
Mon42	6
Mon49	5
Mon9	6
A226	4
A248	5

Marker	Number of alleles
Ag6	6
Hj35	8
M53	4
Mm10.7	4
Mm5.1	4
Ss10.4	5
Ss13.8	6
TGN	5
fs15	3
fs44	5
fs46	3
fs48	3
fs50	4
hic.2.52	8
hic.4.30	9
Ss11.12	10
AHT130	4
Ag8	4
Ss7.1	6
fs41	8
hic.1.95	5

3

Inbreeding depresses altruism in a cooperative society

David A. Wells, Michael A. Cant, Joseph I. Hoffman* & Hazel J. Nichols*

In preparation for Science. *Joint senior author

Abstract:

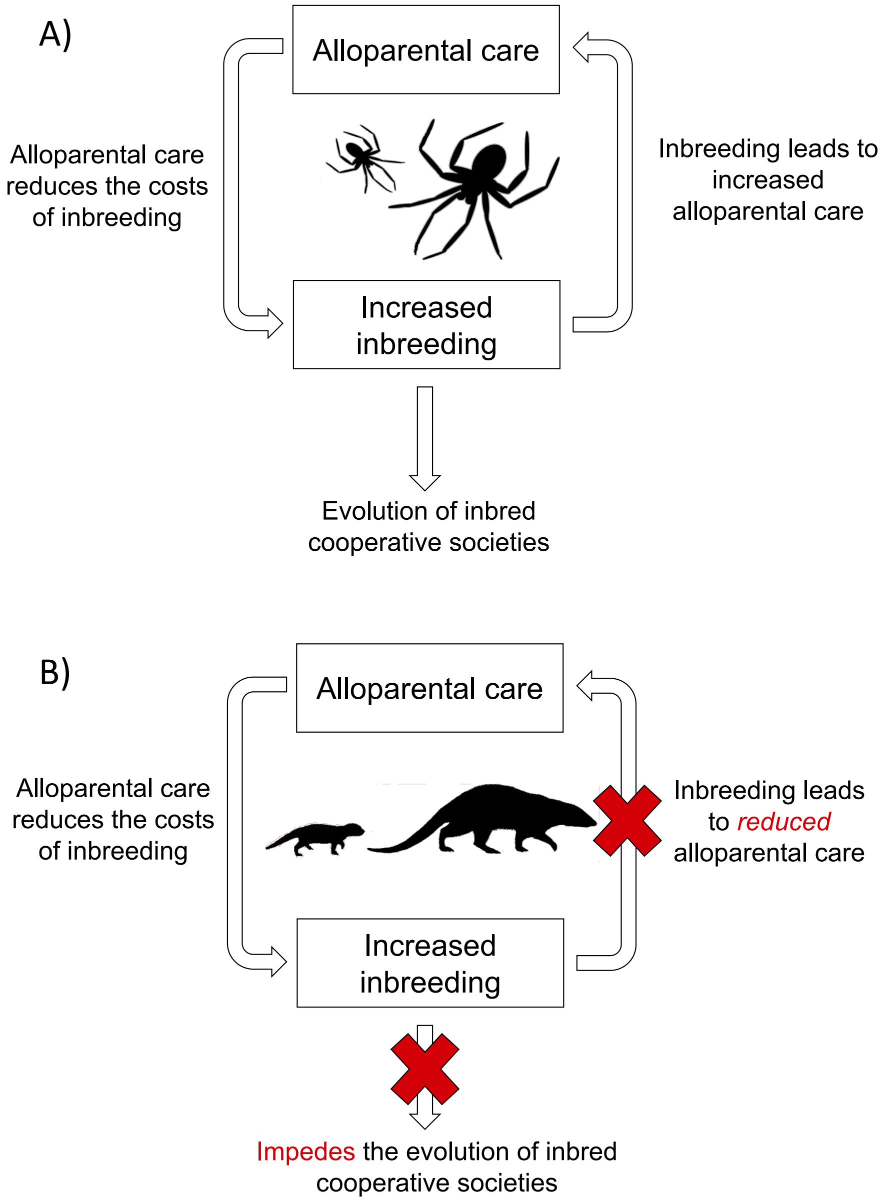
Theory suggests that inbreeding and altruism can be tightly linked. Specifically, altruistic alloparental care received early in life should reduce inbreeding depression and therefore reduce selection to avoid inbreeding. A consequent increase in inbreeding would elevate relatedness levels and may promote further altruistic care, thereby facilitating evolutionary transitions to inbred cooperative societies. However, this hypothesis has not been empirically tested. We therefore analysed 23 years of data from wild banded mongooses to show that altruistic alloparental care does mitigate inbreeding depression for early survival. However, as adults, inbred individuals provide less alloparental care, which should oppose selection for increased inbreeding. Our results suggest that one reason inbred societies are rare in nature is that the protective altruism that enables elevated levels of inbreeding is itself reduced by inbreeding depression.

Main text:

Habitual inbreeding is rare in the animal kingdom due to the ubiquitous negative impact of inbreeding on fitness (known as inbreeding depression) which results in strong selection for inbreeding avoidance (1). However, inbreeding occurs as a regular part of the mating system in a number of species (2), raising the question of why such systems evolve. Theory predicts that habitual inbreeding should be favoured when inbreeding depression is low (3,4). This is because inbreeding can provide relatives with additional breeding opportunities, resulting in increased inclusive fitness (3,5). Furthermore, because inbreeding increases relatedness among individuals, in theory it can and favour the spread of altruism (6–8). But under what circumstances can inbreeding depression be reduced sufficiently to allow transitions to inbred mating systems?

A key factor that could modulate this transition is the provision of early-life care (9). By protecting offspring from environmental stressors, care may mitigate inbreeding depression (10,11) and lead to the higher levels of inbreeding and relatedness that favour the evolution of altruism (8,12,13) (Fig. 1A). This 'protective altruism' hypothesis has been proposed to explain the evolution of the inbred cooperative systems observed in social spiders, where dispersal is rare, individuals habitually breed with close relatives, and levels of cooperation including alloparental care are extremely high (2). It may also play a role in explaining the relatively high levels of inbreeding tolerance found in a handful of cooperatively breeding vertebrates (14). However, so far the possibility that care buffers against inbreeding depression has only been tested empirically in a laboratory population of burying beetles (15), while no studies have investigated how much alloparental care inbred individuals provide. Moreover, both inbreeding depression and the benefits of care are expected to be higher in the wild due to greater levels of environmental stress. Consequently, the protective altruism hypothesis needs to be fully evaluated in a wild animal society in order to better understand the conditions under which inbred cooperative mating systems might evolve.

Fig. 1. Schematic illustrating (A) the 'protective altruism' hypothesis; and (B) constraints observed in a wild banded mongoose population that may oppose evolution towards inbred cooperative societies. In social spiders, alloparental care has been hypothesised to decrease the costs of inbreeding, which should favour an increase in inbreeding and the evolution of altruism by increasing levels of relatedness (9). In the banded mongoose alloparental care does decrease the costs of inbreeding but inbreeding decreases the level of alloparental care. This should limit the tolerable level of inbreeding and prevent transitions to the inbred cooperative societies seen in the social spiders.



A critical but so far untested assumption of the hypothesis that early-life care can lead to the evolution of inbred social systems is that care itself does not suffer inbreeding depression (15). Parental care has been shown to suffer inbreeding depression in some species (16,17), possibly because inbred individuals have fewer resources available to spend on care. However, the situation may be different in the case of alloparental care. In cooperative species, there is often intense competition for rare breeding opportunities (18). If inbred individuals are less likely to be successful competitors, they may direct care towards relatives' offspring as an alternative method to maximise their inclusive fitness. Under such circumstances, high levels of inbreeding may even increase the total amount of care provided to offspring and thereby drive transitions to inbred cooperative mating systems (9). Such transitions may be particularly likely if care is targeted towards inbred offspring, who will have the lowest fitness in the absence of care (19).

We test three predictions resulting from the hypothesis that alloparental care may facilitate evolutionary transitions to inbred cooperative societies; (i) care of offspring reduces inbreeding depression; (ii) care is directed towards inbred individuals; and (iii) care itself is impacted by inbreeding. To address these predictions, we use an exceptionally large dataset (2,023 hours of observational data coupled with genetic data from 1,125 individuals) from a wild population of cooperatively breeding banded mongooses (*Mungos mungo*). This species lives in social groups composed primarily of relatives with multiple breeding males and females (20). Both sexes routinely breed within their natal group, leading to frequent close inbreeding (9% of pups are the product of father-daughter or full-sibling matings (21)) and high variance in inbreeding (22).

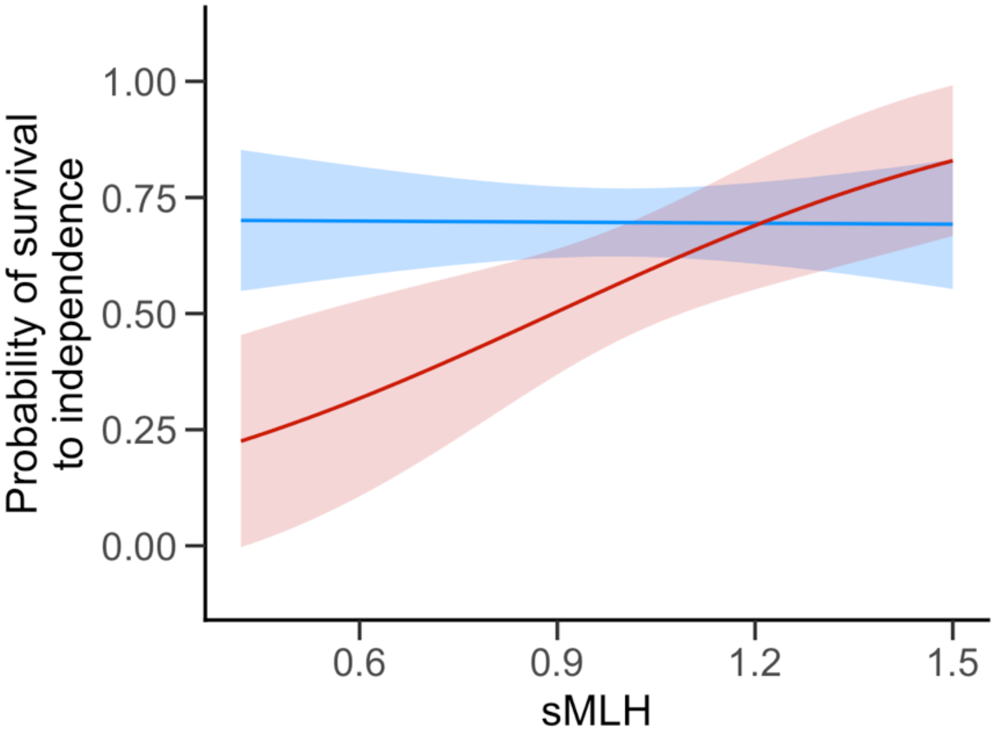
This species shows two distinct forms of offspring care: babysitting and escorting. For the first 30 days after birth, one or more adults remain at the den to protect the communal litter while the rest of the group forages. This form of care is termed babysitting and is not directed towards specific individuals (Fig. 2A). Once pups are mature enough to accompany the group on foraging trips, many form exclusive one-to-one relationships with an adult who feeds, carries, grooms and protects them from predators (23,24). This form of care is unique to the banded mongoose and is termed escorting (Fig. 2B). The escorting relationship lasts for approximately two months (until the pup is ~90 days old). Pups vary in the amount of care they receive from escorts (24) and some pups were not escorted at all (Fig. S1B), instead moving between different adults while begging for food. Adults also vary in their contributions to care and some individuals provide no care (Fig. S1C and D). This naturally occurring variance in inbreeding and care makes the banded mongoose system ideally suited to investigating evolutionary feedbacks between alloparental care and inbreeding in the wild.

Fig. 2. Banded mongoose caring behaviour. (**A**) babysitting, where one or more adults remain at the den to protect all pups in the communal litter; and (**B**) escorting, where an adult provides one-to-one care for a single pup over a two-month period. Photo credits: David Seager and Hazel Nichols.

Using generalised linear mixed models (GLMMs), we first test the prediction that care provided by escorts reduces inbreeding depression for two fitness traits in pups: survival to, and weight at nutritional independence (see Table S1 and Materials and Methods for details). We focus on escorting for this analysis because it is targeted towards specific individuals, whereas babysitting is directed towards entire litters. To quantify inbreeding, we use standardised multilocus heterozygosity (sMLH) measured at 35–43 microsatellite loci.

We show that severe inbreeding depression in pup survival is mitigated by care provided by escorts (sMLH:care ß = -4.23, $2LL_1 = 6.29$, $p = 0.012$, Fig. 3 and Table S2). We also find a borderline non-significant trend for care to reduce inbreeding depression for pup weight (sMLH:care ß = -0.27, $2LL_1 = 2.90$, $p = 0.088$, Fig. S2 and Table S3). These results demonstrate that early-life care can substantially reduce inbreeding depression in a wild population and suggest that estimates of the strength of inbreeding depression may be severely downwardly biased when a protective effect of care is not accounted for.

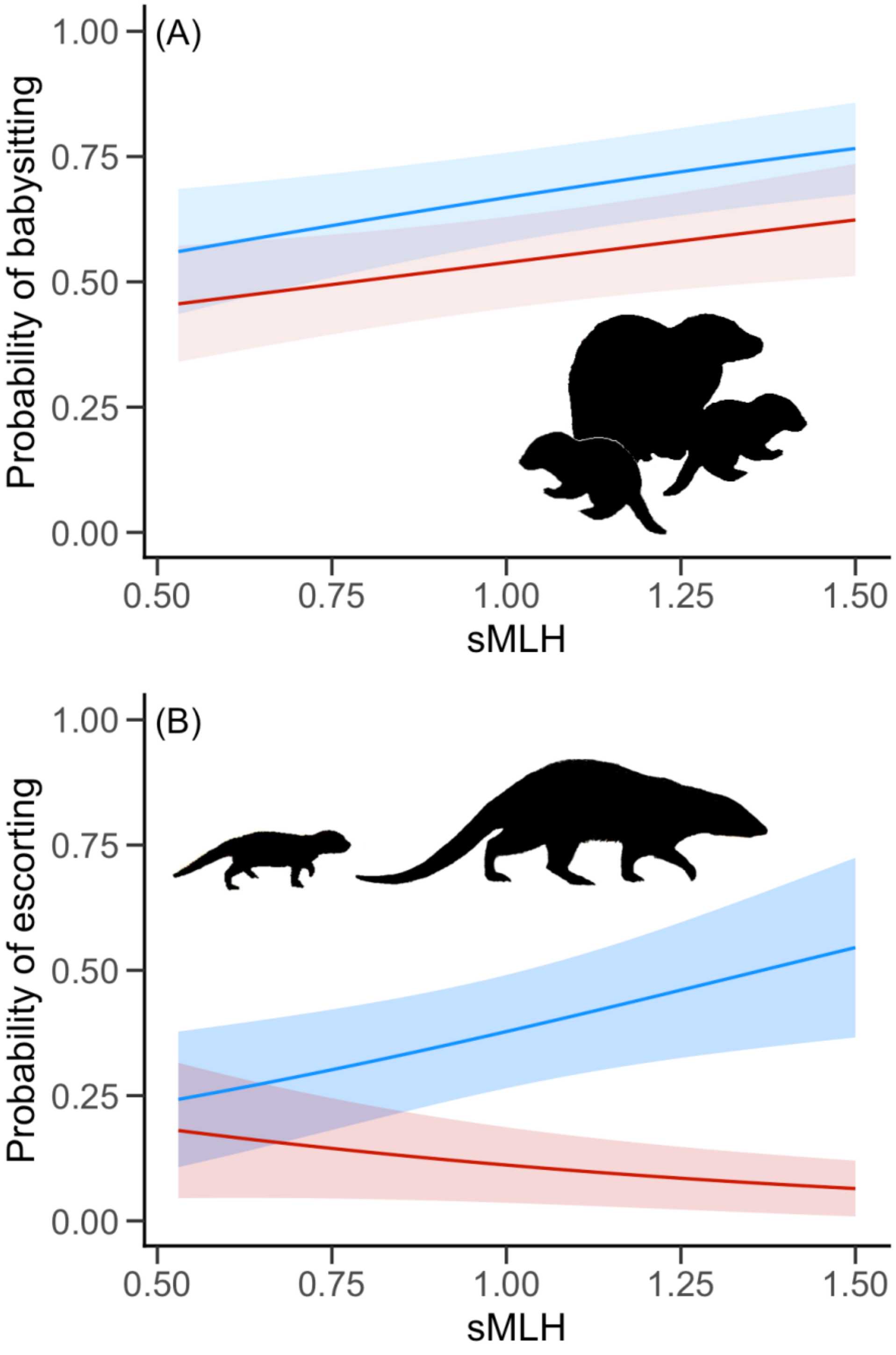
Fig. 3. Care provided by escorts mitigates inbreeding depression for early survival. Shown is the probability of pup survival to 90 days for offspring receiving no escorting care (in red) versus offspring receiving the average non-zero amount of escorting care (i.e. escorting was observed during 62% of observations, in blue). Trend lines show predicted values from the fitted model and the shaded regions show 95% CIs.



Second, we test the prediction that care is directed towards inbred pups, as these pups would benefit the most from care. However, we find no evidence that inbred pups received more care than outbred pups (sMLH ß = 0.15, $2LL_1$ = 0.04, p = 0.84, Table S4). Failure to direct escorting towards inbred pups means that care cannot fully mitigate inbreeding depression at the population level. Consequently, the full benefits of alloparental care may not be realised, weakening selection for inbreeding.

Finally, we test whether the inbreeding level of adults influences the amount of cooperative care they provide. We find that inbreeding reduces both babysitting and escorting. Specifically, relatively inbred individuals of both sexes are less likely to babysit (males: sMLH ß = 0.97, $2LL_1$ = 8.86, p = 0.003; females: sMLH ß =0.69, $2LL_1$ = 4.73, p = 0.030, Fig. 4A, Table S5A and B). For those individuals that do babysit a litter, inbred males provide less care (sMLH ß = 0.42, $2LL_1$ = 5.62, p = 0.018, Table S5C) whilst inbreeding does not significantly affect the amount of babysitting provided by females (sMLH ß = -0.12, $2LL_1$ = 1.04, p = 0.308, Table S5D). Inbred males are also less likely to escort a pup (sMLH ß = 1.36, $2LL_1$ = 5.23, p = 0.022, Fig. 4B, Table S6A) while the likelihood of escorting increases marginally with inbreeding in females (sMLH ß = -1.20, $2LL_1$ = 4.37, p = 0.037, Fig. 4B, Table S6B). For individuals that escort a pup, inbreeding does not influence the amount of time spent escorting in either sex (males: sMLH ß = 0.30, $2LL_1$ = 0.53, p = 0.47; females: sMLH ß = -0.17, $2LL_1$ = 0.10, p = 0.76, Table S6C and D). As more than two thirds of all care is provided by males, the overall effect of inbreeding is to reduce the total amount of care provided to offspring. Low levels of escort care increase pup mortality (25,26) and litters repeatedly left without a babysitter never survive as they are killed by predators or rival groups (23). Thus, our results suggest that inbreeding depression is not limited to inbred individuals but can affect all individuals in a social group via its effects on alloparental care.

Fig. 4. Inbreeding reduces the provision of alloparental care. Shown is the probability of providing (**A**) babysitting care and (**B**) escorting care, conditional on sMLH and plotted separately for males (in blue) and females (in red). Trend lines show predicted values from the fitted models and the shaded regions refer to the associated 95% CIs. Inbred individuals of both sexes were less likely to be observed babysitting, whereas escorting was negatively associated with escorting in males but positively associated with escorting in females (see main text for details).



Our study reveals feedback between early-life care and inbreeding depression (Fig. 1B). We demonstrate empirically that cooperative care can mitigate inbreeding depression for survival, which may theoretically facilitate the evolution of inbred cooperative societies (9). However, we also show that care itself suffers from inbreeding depression, which will act as a constraint opposing such transitions. This constraint could explain why inbreeding is rare among species with alloparental care. In this species, inbreeding depression is not limited to inbred individuals but also impacts all group-members belonging to subsequent generations, regardless of how inbred those individuals are. Such cross-generational effects may serve to magnify inbreeding depression.

To conclude, high levels of inbreeding and relatedness increase genetic homogeneity, which has been proposed as one of the main factors that can cohere societies into a new level of biological organisation (27,28). However, this argument assumes that genetic homogeneity *per se* has no other cost to cooperation. Our results suggest that, in animal societies, inbreeding may counter selection for helping. The extent to which mechanisms can evolve to escape this retarding influence of inbreeding on cooperation may have important implications for social cohesion and altruism in wild populations.

Acknowledgments

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Supplementary material:

Materials and methods

Study site and data collection

We used data collected between January 1993 and July 2016 inclusive as part of an ongoing long-term study of a wild population of banded mongooses (*Mungos mungo*) in Queen Elizabeth National Park, Uganda (0°12´S, 27°54´E). The study site comprises approximately 10km^2 of savannah on and around the Mweya Peninsula, which contains roughly 250 individual banded mongooses at any one time belonging to 10–12 social groups. At the centre of the study site is a weather station, which collects daily rainfall measurements.

All individuals in the population can be identified on sight due to a unique fur shave or dye pattern (l'Oreal, UK) or colour-coded plastic collar. These markings are maintained by trapping all individuals in the population every 3–6 months as described in (29). Body mass (g) is measured without trapping as individuals are habituated to step onto portable scales for a small milk reward.

One or two adults in each group are fitted with a 26g radio collar ($<$2% of body mass, Sirtrack Ltd., New Zealand) with a 20cm whip antenna (Biotrack Ltd., UK), which allows groups to be located. Groups are habituated to human observation (within 5m) and are visited every 1–3 days to collect detailed behavioural and life history data.

Quantifying alloparental care given and received

Reproduction is highly synchronised within social groups, with up to 13 females (median = 3) giving birth together in an underground den, often on the same night (30). This leads to large litters of mixed parentage that are raised by multiple group members including parents and non-parents (24). Pups do not leave the den during their first month of life, and during this period one or more adults stay at the den while the rest of the group leaves to forage. We refer to this form of pup care as 'babysitting'. Babysitting is important for litter survival as litters without babysitters are vulnerable to predators or infanticide by rival groups (23). Babysitters are identified by either being observed at the den while the rest of the group forages at least 100m away, or by their absence from the group on foraging trips (29).

When pups are aged between ~30 and ~90 days, they accompany the group on foraging trips and are fed by adult group-members. Pups usually form one-to-one relationships with a particular adult, termed an escort, who feeds, grooms, carries, and protects the pup. Pups vary in the amount of time they spend with an escort, with some pups receiving no escorting care (Fig. S1B) and instead moving between different adults in quick succession while begging for food. Similarly, adults vary in the amount of escorting care they provide, and many adults do not escort a pup (Fig. S1D). Escorting is not preferentially directed towards close kin (24) and individuals that have not reproduced are some of the primary providers of care (23). During the escorting period, groups are visited once or twice per day to record escorting behaviour. Escorting is very conspicuous and is therefore easy to identify visually. Adults are classified as escorting a pup if they spend more than half of a given 20 minute observation period within 0.3m of the focal pup (24).

Genetic data

The first time individuals are captured, they are fitted with a PIT tag (TAG-P-122IJ, Wyre Micro Design, UK) for permanent identification and their sex is determined through visual inspection. For genetic analysis, a 2 mm tissue sample is taken from the tail tip using surgical scissors and afterwards a dilute solution of potassium permanganate is applied to minimize the risk of infection.

From 1993–2016, we collected a total of 1,125 tissue samples. These were genotyped for 35–43 microsatellite loci and the resulting data were used to determine parentage as described in (22,31). Pairwise relatedness values were derived from a maximal nine-generation deep pedigree (22,31). We then quantified inbreeding directly from the genetic data by calculating standardised multilocus heterozygosity (sMLH) within the R package inbreedR (32). We used this measure for further analyses, as we have previously shown that for some traits sMLH outperforms pedigree *f* at capturing variance in inbreeding and quantifying inbreeding depression in our mongoose population (22,33), and partly because the pedigree is much smaller than the number of genotyped individuals after filtering for depth (all four grandparents present) (22,31).

Ethical statement

Our research was carried out under licence from the Uganda National Council for Science and Technology, and all procedures have been approved by the Uganda Wildlife Authority. All research procedures adhere to the ASAB Guidelines for the Treatment of Animals in Behavioural Research and Teaching and have been approved by the Ethical Review Committee of the University of Exeter. Our trapping procedure has been used over 8,000 times, and tissue samples have been taken from over 1,000 individuals with no adverse effects.

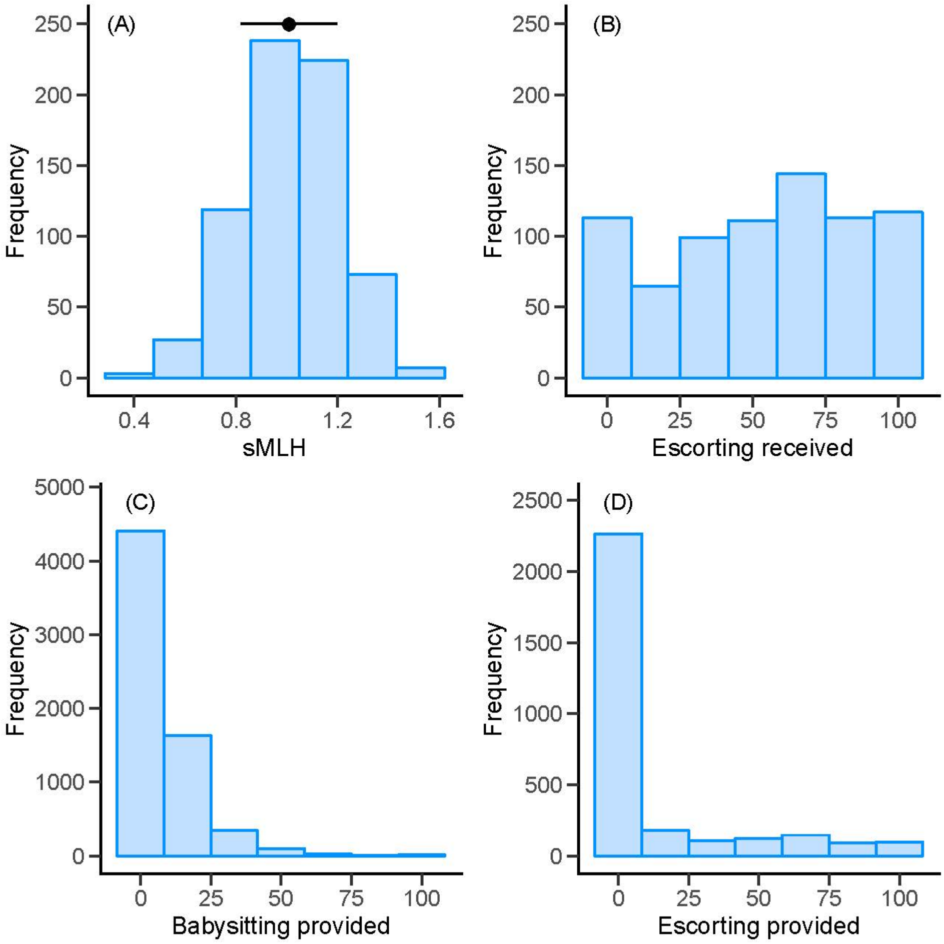
Statistical analysis

We constructed generalized linear mixed models (GLMMs) and generalised additive mixed models (GAMMs) in the R packages lme4 (34) and gamm4 (35) respectively to investigate (i) whether care reduces inbreeding depression; (ii) whether care is directed towards inbred pups; and (iii) whether the provisioning of care is itself impacted by inbreeding. Details of the specific models are provided below. All of the variables that we analysed are defined in Table S1 and the distributions of key variables across our dataset are shown in Fig. S1. All of our models were checked for colinearity of predictor variables and validated by inspecting histograms of the residuals and plots of the residuals against predictor variables.

Table S1. Definitions and units of variables analysed in our statistical models.

Variable	Definition
Survivorship to nutritional independence	Pup survival to 90 days (1 = survived, 0 = died).
Weight at nutritional independence	Weight (g) at as close to 90 days as possible, over 70% of measurements were taken within a week of 90 days (range 61–119 days old).
Escorting care received	The proportion of observation sessions that a focal pup was observed to have an escort.
Babysitting care provided	The proportion of observation sessions that a focal adult was observed to babysit a litter.
Escorting care provided	The proportion of observation sessions that a focal adult was observed to escort a pup.
Inbreeding	Standardised multilocus heterozygosity (sMLH) at 35–43 microsatellite loci.
Rainfall	Rainfall (mm) across the relevant time period (see descriptions of each model for details). Rainfall influences food abundance/quality and therefore impacts on growth, survival and behaviour.
Sex	Whether the focal pup or potential carer is male or female.
Age	Age (days) of the focal pup or potential carer.
Litter size	Number of pups present in the communal litter at emergence (at 30 days).
Parentage	Whether or not a potential carer was assigned as a mother or father to pups in the communal litter (1 = yes, 0 = no) using genetic data.
Relatedness	The average pedigree relatedness of the focal potential carer to other potential carers present in the social group.

Fig. S1. The distribution of key variables analysed in our study. (**A**) individual inbreeding levels, quantified as standardised multilocus heterozygosity (sMLH) at 35–43 microsatellites. Mean sMLH +/- 1 SD (0.19) is shown above the histogram; (**B**) escorting care received, quantified as the percentage of sessions that a given pup was sighted together with an escort (**C**) babysitting care provided, quantified as the percentage of observation sessions that a potential carer was observed to babysit a litter, and (**D**) escorting care provided, quantified as the percentage of observation sessions that a potential carer was observed escorting a pup.



Does care reduce inbreeding depression?

We investigated the impact of escorting care on inbreeding depression for two early-life fitness traits: survival to, and weight at, nutritional independence (which occurs at approximately 90 days of age). Note that we could not investigate a similar impact of babysitting care as it was not possible to determine the survivorship or weight of pups prior to emergence from the den.

The first of these models investigated pup survival to 90 days, which was expressed as a binary response variable. We fitted as predictor variables the amount of escorting care received, pup sMLH, and an interaction between escorting and sMLH. Models with and without the interaction term were compared using a likelihood ratio test to determine its significance. Average rainfall over the 30 days prior to birth was also included as a predictor variable because a recent study found it to be strongly associated with pup survival (22). To account for non-independence among pups the identity of the social group and litter that the pup was present in were included as random effects. The full results of this model are described in Table S2.

Table S2. Results of the GLMM of pup survival to independence. Shown are model estimates and standard errors together with log likelihood ratios (2LL) and associated *p*-values obtained by removing the term in question from the full model. The model was based on data from a total of 776 pups from 142 litters in 11 groups.

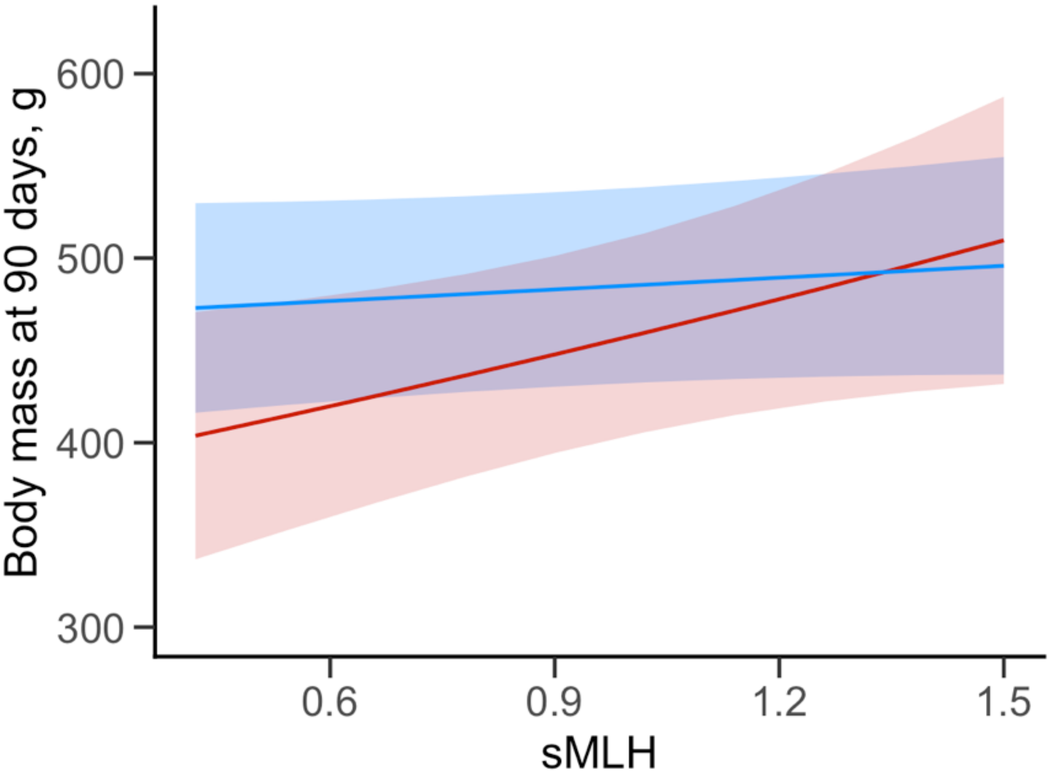
	Estimate	Std. Error	2LL	*p*-value
(Intercept)	-3.047	1.130		
Rainfall	0.266	0.103	6.735	0.009
Escorting:sMLH	-4.229	1.707	6.292	0.012
Escorting	5.112	1.765		
sMLH	2.607	1.059		

The second of these models investigated pup body mass at nutritional independence. For logistical reasons, it was not always possible to measure mass at exactly 90 days, so we used the closest available measurement (see Table S1 for details). To account for growth over this period, age at weighing (in days) was included in the model as a covariate. As above, the amount of escorting care received, pup sMLH, and an interaction between escorting and sMLH were included as predictor variables. We then tested whether care reduced inbreeding depression for body mass by comparing models with and without an interaction between care and sMLH using a likelihood ratio test. Average rainfall over the first two months of an individual's life was also included as a predictor variable due to the importance of early life rainfall on growth (26). To enable this model to converge, both rainfall and age at weighing were standardised by subtracting the mean and dividing by the standard deviation. To account for non-independence among pups, both social group and litter were included as random effects. We modelled pup weight with a negative binomial error distribution to account for heterogeneity in residuals. The full results of this model are described in Table S3 and plotted in Fig. S2.

Table S3. Results of the GLMM of pup weight at independence. Shown are model estimates and standard errors together with log likelihood ratios (2LL) and associated *p*-values obtained by removing the term in question from the full model. The model was based on data from a total of 443 pups from 120 litters in 10 groups.

	Estimate	Std. Error	2LL	*p*-value
(Intercept)	5.757	0.113		
Escorting:sMLH	-0.272	0.159	2.902	0.088
sMLH	0.216	0.102		
Escorting	0.365	0.165		
Age at weighing	0.15	0.01	188.959	<0.001
Rainfall	0.067	0.018	13.208	<0.001

Fig. S2. A non-significant trend for care to reduce inbreeding depression for body mass. Shown is body mass at 90 days for offspring receiving no escorting care (in red) versus offspring receiving the average non-zero amount of escorting care (i.e. escorting was observed during 63% of observations, in blue), conditional on sMLH. Trend lines show predicted values from the fitted model and the shaded regions refer to the associated 95% CIs.



Do inbred pups receive more care?

We tested whether escorting care is preferentially directed towards inbred pups. This analysis focused on escorting, because this behaviour is directed towards specific individuals, whereas babysitting is directed towards an entire litter of multiple pups which are not all equally inbred. The amount of escorting care received was fitted as a response variable in a binomial GLMM. Predictor variables were sMLH, sex, litter size, and the average rainfall over the 60 day escorting period and the 14 days before it (24,36). Rainfall was standardised by subtracting the mean and dividing by the standard deviation.

As in previous models, group and litter were included as random effects. The full results of this model are presented in Table S4.

Table S4. Results of the GLMM investigating whether escorting care is directed towards inbred pups. Model estimates and standard errors are shown together with log likelihood ratios (2LL) and associated *p*-values obtained by removing the term in question from the full model. The model was based on data from a total of 762 pups from 138 litters in 11 groups.

	Estimate	Std. Error	2LL	*p*-value
(Intercept)	0.968	0.239		
Sex (male)	0.094	0.052	3.173	0.075
Rainfall	0.041	0.077	0.29	0.59
sMLH	0.03	0.147	0.042	0.837
Litter size	-0.066	0.019	11.876	<0.001

Is the amount of care provided by adults affected by inbreeding?

We tested whether inbreeding affects the extent to which potential carers provide alloparental care in the forms of babysitting and escorting. These analyses were implemented using GAMMs. Individuals were identified as potential carers if they were at least six months old at the start of the care period and were in the same social group as the litter. Contributions to babysitting and escorting showed zero inflation (Fig. S1C and D). To account for this we constructed two models for each type of care; (i) a binary model analysed whether individuals were observed to provide care to a litter on at least one occasion (1 = care provided, 0 = no care provided); (ii) for those individuals that were observed to provide care, their contributions to care were fitted as a response term in a binomial model using the cbind function in R (observed caring x times out of n observations). We ran two models rather than using a single binomial model accounting for zero-inflation in order to incorporate non-monotonic response variables (see below).

We fitted sMLH as an explanatory variable, together with other variables that have been shown to affect the provision of care in previous studies (age, parentage, rainfall, relatedness and litter size, see Table S1 for definitions) (24,36). The relationship between age and care is non-monotonic, increasing sharply in early life before levelling off or decreasing. To account for this, we modelled age using a thin plate regression spline. Rainfall (mm) was taken as the average over the 30 or 60 day care period and the 14 days prior to it for babysitting and escorting respectively, and in both cases it was standardised by subtracting the mean and dividing by the standard deviation. Litter size was used in models of escorting but not of babysitting because it is not possible to count the number of pups in the litter before they emerge from the den. To account for non-independence, the identity of the social group, litter and individual were fitted as random effects. We constructed separate models for males and females because the extent to which the two sexes provide care is strongly influenced by their different life histories (29). Furthermore, rainfall influences the caring behaviour of female breeders and non-breeders differently (36). We therefore fitted an additional interaction between rainfall and parentage. The models of babysitting behaviour are presented in Table S5 and Table S6 describes the models of escorting behaviour.

Table S5. Results of GAMMs investigating whether the amount of babysitting care provided is affected by inbreeding. Models with binary response variables (**A** and **B**) investigated whether or not individuals babysat the focal litter, while models with binomial response variables (**C** and **D**) investigated the amount of babysitting care provided. Shown are model estimates and standard errors together with log likelihood ratios (2LL) and associated *p*-values obtained by removing the term in question from the full model. Estimated degrees of freedom (EDFs) provide a measure of the non-linearity of the smoother term. The male models (**A** and **C**) were based on 4,006 samples where each sample represents all care provided to a specific litter by a given individual, of these 2,270 included some care and so were included in the binomial model. The male dataset included 395 potential babysitters of 389 litters in 14 social groups. The female models (**B** and **D**) were based on 2,486 samples of which 1,198 included some care. The female dataset included 292 potential babysitters over 377 litters in 14 groups.

Model	Predictor	Coefficient	SE	2LL	*p*-value	EDF
(**A**) Male binary	(Intercept)	-0.121	0.396			
	Parentage	0.198	0.16	1.482	0.223	
	Rainfall	0.341	0.084	16.174	<0.001	
	sMLH	0.973	0.323	8.862	0.003	
	Relatedness	-2.613	0.762	11.573	<0.001	
	Age	2.248	0.768	157.41	<0.001	7.226
(**B**) Female binary	(Intercept)	-0.54	0.388			
	Parentage	0.166	0.146	1.247	0.264	
	Rainfall	0.158	0.089	3.096	0.078	
	sMLH	0.686	0.315	4.729	0.030	
	Relatedness	-0.997	0.865	1.299	0.254	
	Age	0.724	0.65	25.222	<0.001	5.658
(**C**) Male binomial	(Intercept)	-2.364	0.195			
	Parentage	-0.168	0.047	12.84	<0.001	
	Rainfall	0.013	0.023	0.329	0.566	
	sMLH	0.419	0.176	5.618	0.018	
	Relatedness	0.596	0.341	3.078	0.079	
	Age	1.676	0.286	151.3	<0.001	7.613
(**D**) Female binomial	(Intercept)	-1.947	0.218			
	Parentage:Rainfall	-0.105	0.049	4.845	0.0278	
	Parentage	-0.070	0.055			
	Rainfall	-0.016	0.033			
	sMLH	-0.119	0.159	1.039	0.308	
	Relatedness	0.458	0.406	1.243	0.265	
	Age	0.039	0.028	1.951	0.377	5.65

Table S6. Results of GAMMs investigating whether the amount of escorting care provided is affected by inbreeding. Models with binary response variables (A and B) investigated whether or not individuals escorted the focal pup, while models with binomial response variables (C and D) investigated the amount of escorting care provided to the focal pup. Shown are model estimates and standard errors together with log likelihood ratios (2LL) and associated *p*-values obtained by removing the term in question from the full model. Estimated degrees of freedom (EDFs) provide a measure of the non-linearity of the smoother term. The male models (A and C) were based on 1,804 samples where each sample represents all care provided to a specific litter by a given individual, of which 540 included some care and so were included in the binomial model. The male dataset included 310 potential babysitters of 156 litters in 9 social groups. The female models (B and D) were based on 1,206 samples or which 244 included some care. The female dataset included 233 potential babysitters over 156 litters in 10 groups.

Model	Predictor	Coefficient	SE	2LL	*p*-value	EDF
(**A**) Male binary	(Intercept)	-3.439	0.738			
	Parentage	0.466	0.216	4.630	0.031	
	Rainfall	-0.356	0.115	9.834	0.002	
	Litter size	0.168	0.028	33.395	<0.001	
	sMLH	1.363	0.595	5.225	0.022	
	Relatedness	-3.065	1.474	4.414	0.036	
	Age	3.542	1.529	106.06	<0.001	7.206
(**B**) Female binary	(Intercept)	-1.942	0.776			
	Parentage	1.255	0.23	32.256	<0.001	
	Rainfall	-0.351	0.149	5.807	0.016	
	Litter size	0.161	0.035	18.616	<0.001	
	sMLH	-1.196	0.557	4.369	0.037	
	Relatedness	-2.251	1.585	2.070	0.150	
	Age	5.839	1.861	29.894	<0.001	6.682
(**C**) Male binomial	(Intercept)	-0.955	0.513			
	Parentage	-0.065	0.107	0.369	0.544	
	Rainfall	-0.147	0.103	1.975	0.160	
	Litter size	-0.01	0.023	0.194	0.660	
	sMLH	0.304	0.415	0.528	0.467	
	Relatedness	4.521	1.022	19.428	<0.001	
	Age	-0.272	0.207	9.811	0.007	2.749
(**D**) Female binomial	(Intercept)	0.298	0.669			
	Parentage:rainfall	-0.505	0.164	9.659	0.002	
	Parentage	0.136	0.16			
	Rainfall	0.39	0.172			
	Litter size	-0.062	0.032	3.836	0.050	
	sMLH	-0.17	0.544	0.097	0.756	
	Relatedness	1.839	1.329	1.896	0.169	
	Age	1.447	0.721	10.892	0.004	4.729

Could our results be confounded by covariance between inbreeding and the number of observations of a given individual?

It is conceivable that a relationship between inbreeding and care received or provided could result as an artefact of covariance between inbreeding level and the number of times an individual was observed during the period of care. Such a relationship could arise, for example, if inbred individuals are more likely to die during the caring period. To test for this possibility, we constructed three separate zero-truncated Poisson GLMMs in which the respective response variables were (i) the number of times a pup was observed, (ii) the number of times a potential babysitter was observed; and (iii) the number of times a potential escort was observed. sMLH was included as the sole predictor variable together with individual, litter and social group as random effects.

We found that sMLH was not significantly associated with any of the three variables (*escorting care received*: sMLH ß = 0.53, $p = 0.51$; *babysitting care provided*: males sMLH ß = 0.001, $p = 0.96$, females sMLH ß = 0.004, $p = 0.89$; *escorting care provided:* males sMLH ß < 0.001, $p = 0.99$, females sMLH ß > -0.001, $p = 0.99$). Our results are therefore not confounded by covariance between the number of times a focal individual is observed and the level of inbreeding of that individual.

4

Adaptive plasticity of extra-group breeding according to risk of inbreeding

David A. Wells, Michael A. Cant, Joseph I. Hoffman* & Hazel J. Nichols*

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Abstract

Plasticity can allow animals to adaptively vary their behaviour according to changing conditions. An individual's fitness depends on their ability to perceive and respond to environmental cues and perform the optimal behaviour according to current conditions. Despite a fundamental role in fitness, alternative breeding behaviours, their triggers, and their consequences all remain poorly understood. We used 24 years of breeding behaviour and a nine-generation deep pedigree in the banded mongoose to investigate changes in social groups and their effect on within- vs extra-group breeding behaviour in females. We found that females were more likely to conceive to extra-group males when the risk of within-group inbreeding was high. However, their breeding behaviour was informed by several societal proxies instead of within-group relatedness directly. Extra-group mates were unrelated, so extra-group breeding resulted in outbred offspring; but despite this, the majority broods were sired by within-group males and 20% of those were at least moderately inbred ($f_b \geq 0.125$). Frequent incest implies that there is a high cost to extra-group breeding which selects for inbreeding tolerance despite inbreeding depression. In conclusion, inbreeding avoidance is constrained by costly consequences of these behaviours and the accuracy of environmental proxies.

Introduction

Breeding decisions have important consequences for all levels of evolution, affecting gene-flow as well as individual fitness and therefore selection pressures. Through behavioural plasticity, individuals can display alternative breeding decisions in different conditions; ostensibly these decisions are an adaptive response to short term environmental changes. Despite their importance, the triggers and fitness consequences of these decisions are far from clear. One of the best studied breeding decisions in this context is extra-pair breeding in birds, but despite multiple meta-analyses there is no consensus on whether this behaviour is generally adaptive or the non-adaptive result of genetic correlations (Arct, Drobniak, & Cichoń, 2015; Hsu, Schroeder, Winney, Burke, & Nakagawa, 2015).

An important selection pressure thought to shape the evolution of breeding behaviour is inbreeding depression (Pusey & Wolf, 1996). The offspring of close relatives are largely homozygous and suffer low fitness, known as inbreeding depression, due to the expression of deleterious recessive alleles and a lack of heterozygote advantage (Charlesworth & Willis, 2009). This phenomenon has been documented across sexual animals and plants (Keller & Waller, 2002) causing a reduction in fitness traits throughout an individual's life. This widespread distribution of inbreeding depression is believed to have selected for parents to breed preferentially with non-relatives, known as inbreeding avoidance, in many species (Pusey & Wolf, 1996).

Although inbreeding avoidance behaviours can produce higher quality offspring they may also incur a cost. If this cost is sufficient to outweigh any fitness gain then inbreeding avoidance behaviour will not be favoured. For example (Keller & Arcese, 1998) proposed that the opportunity costs of rejecting related mates prevented the evolution of inbreeding avoidance in the Mandarte island song sparrow. Furthermore, simulations show that associated opportunity costs can strongly select against inbreeding avoidance when breeding opportunities are limited (Kokko & Ots, 2006).

The costs of inbreeding avoidance are unlikely to be fixed, instead they will probably vary with social and environmental conditions. Individuals could maximise their fitness by plastically altering their behaviour according to the current cost-benefit ratio. Similarly, the risk of inbreeding when mating randomly may also vary, in which case the benefits of actively avoiding inbreeding will change too. When potential mates are related, the risk of inbreeding is high but over time this risk can change, possibly due to turnover in breeding individuals. When the risk of breeding with a relative is low there is little to be gained from inbreeding avoidance compared to mating randomly with respect to relatedness (Jamieson, Taylor, Tracy, Kokko, & Armstrong, 2009). Therefore, individuals may plastically alter their breeding behaviour and only pay the costs of inbreeding avoidance when the risk of inbreeding is otherwise high. For example female European badgers are more likely to produce extra-group offspring when within group relatedness is high (Annavi et al., 2014).

In order to exploit the benefits of plasticity, individuals must be able to accurately identify the optimal behaviour based on environmental cues. The consequences of failing to do so are apparent where environmental change has caused mismatches between environment and plastic traits (Bonamour, Chevin, Charmantier, & Teplitsky, 2019). As is common in phenological examples, breeding decisions may not be based upon the fundamentally important variable but a suitable proxy (Bonamour et al., 2019). For illustration, rejecting a sibling as a potential mate may be based upon familiarity instead of a direct estimate of genetic relatedness (Ihle & Forstmeier, 2013).

A complete understanding of these plastic breeding behaviours requires simultaneous consideration of 1) the relevant environmental changes, 2) their effect on breeding behaviour, and 3) the consequences of the different behaviours in different conditions. In sufficiently understood systems we advocate structural equation modelling (sometimes called confirmatory path analysis) for this task as all three aspects can be evaluated in a single statistical structure. In this study we focus on piecewise structural equation modelling which, in essence, joins generalised linear mixed models (GLMMs) together with an explicitly presumed direction of causation (Shipley, 2009). These models allow us to ask if variables affect outcomes directly or indirectly through their effects on other variables. In a structural equation model, variables without an explicit relationship are believed to be independent after accounting for the specified pathways, and this independence claim is also tested. Finally, the goodness-of-fit of the overall model is calculated in order to assess our understanding of the complete system rather than an individual part.

We use piecewise structural equation modelling to investigate societal changes, their effect on female extra-group breeding, and ultimately the inbreeding status of offspring in the banded mongoose (*Mungos mungo*). The banded mongoose is a small (~1-2Kg) mammal which lives in cooperative social groups of approximately 20 adults plus offspring (Cant, Nichols, Thompson, & Vitikainen, 2016). Unusually among cooperatively breeding mammals, there is relatively low reproductive skew as there is no single dominant breeding male or female; instead, multiple males (3-7) and females (1-5) within each group breed several times a year (Cant et al., 2016). Breeding is synchronised within groups, with all females entering oestrus in the same week and giving birth on the same day as each other in an underground den (Cant, Nichols, Johnstone, & Hodge, 2014; Cant et al., 2016). The resulting pups are raised in a communal litter and receive care from multiple group members including parents and non-breeders (Cant et al., 2016; Gilchrist & Russell, 2007; Hodge, 2007). Both sexes commonly remain in their natal group for their entire lives; as a result of this philopatry there is strong genetic structure between groups (Nichols, Jordan, Jamie, Cant, & Hoffman, 2012) and within-group breeding often results in inbreeding (Nichols, Cant, Hoffman, & Sanderson, 2014).

Separate social groups aggressively defend territories and violent intergroup interactions account for 15% of deaths with a known cause (Nichols, Cant, & Sanderson, 2015). Despite this violence, 18% of offspring are sired by extra-group males and the mating is believed to occur during intergroup interactions because extra-group matings have been observed during intergroup interactions and females are more likely to conceive to extra-group males after intergroup interactions (Nichols et al., 2015). Inbreeding depression has been identified in several banded mongoose fitness traits (Mitchell, Vitikainen, Wells, Cant, & Nichols, 2017; Sanderson, Wang, Vitikainen, Cant, & Nichols, 2015; Wells, Cant, Nichols, & Hoffman, 2018) and extra-group offspring have been shown to be more heterozygous and more competitive than within-group offspring (Nichols et al., 2015). The occurrence of two clearly distinct breeding decisions (extra- and within-group breeding) with well-defined biologically significant consequences (risk of violence and risk of inbreeding respectively) makes this system well suited to ask how triggers affect breeding decisions and their consequences.

In this study we address several questions in a single statistical framework. 1) How do groups change through time, specifically in size and relatedness between potential breeders? 2) Do these changes affect a female's probability of extra-group breeding, and finally 3) how does within- and extra-group breeding affect offspring inbreeding coefficients?

Methods

Study system

This study was carried out on a wild but habituated population of the banded mongoose on Mweya peninsula in the Queen Elizabeth National Park, Uganda (0°12´S, 27°54´E). The population has been under continuous study since

1995 and at any one time consisted of approximately 250 individuals belonging to roughly 10 social groups. Social groups could be located to determine group composition and observe behaviours because 1-2 individuals per group were fitted with 27g radio collars (<2% of body mass, Sirtrack Ltd., New Zealand) with 20cm whip antennae (Biotrack Ltd., UK). Individuals within the study population could be identified on sight because adults were given a unique shave pattern in their fur and individuals under 6 months were marked with blonde hair dye (L'Oreal, UK). In order to maintain these identification marks all individuals were trapped approximately every three months using Tomahawk traps (Tomahawk Live Trap Co., Tomahawk, WI, USA). Once trapped, individuals were anaesthetised using isoflurane as described in (Hodge, 2007; Jordan, Mwanguhya, Kyabulima, Rüedi, & Cant, 2010). The first time individuals were trapped they were given either a unique tattoo or more recently a subcutaneous pit tag (TAG-P-122IJ, Wyre Micro Design Ltd., UK) to enable permanent identification. For subsequent genetic analysis a 2mm tissue sample was taken from the tip of the tail using sterile surgical scissors and stored in 96% ethanol. Afterwards, a dilute solution of potassium permanganate was applied to the tail to minimise the risk of infection.

Ethical statement

The trapping procedure has been carried out over 8,000 times and tissue samples have been collected from over 1,900 individuals over the course of the project with no adverse effects. All research procedures adhere to the ASAB Guidelines for the Treatment of Animals in Behavioural Research and Teaching and were approved by the Ethical Review Committee of the University of Exeter. The research was carried out under licence from the Uganda National Council for Science and Technology, and all procedures were approved by the Uganda Wildlife Authority.

Genetic analysis

DNA was extracted from the tissue samples using Qiagen® DNeasy blood and tissue kits following the manufacturer's protocol. Over the course of three decades of data collection, genetic technologies have improved and our genotyping protocol was updated to take advantage of these developments. Specifically, microsatellite loci were originally genotyped individually by radioactive incorporation but latterly multiple microsatellite loci were genotyped simultaneously as multiplexes using fluorescently labelled primers. Eight of the 43 loci originally genotyped using radioactive incorporation failed to amplify consistently as part of a multiplex and so were not genotyped in recent years. All samples were therefore either genotyped at 35 or 43 microsatellite loci. The 35 microsatellites which did successfully multiplex were amplified as seven separate multiplexes in PCR reactions using a Type It kit (Qiagen) according to the manufacturer's protocol with an annealing temperature of 57°C and a reaction volume of 12 µL. PCR products were resolved by electrophoresis on an ABI 3730xl capillary sequencer, and allele sizes were scored using GENEMARKER version 1.95 (SoftGenetics, Pennsylvania, USA). For full details of the multiplex genotyping see (Sanderson et al., 2015; Wells et al., 2018) and for the radioactive genotyping see (Nichols, Amos, Cant, Bell, & Hodge, 2010; Nichols et al., 2012).

Parentage assignment

Parentage could not be assigned based on observations because birth is highly synchronised within groups such that multiple females frequently gave birth on the same night in the same underground den (Cant et al., 2016). Therefore, genetic parentage assignment was required to identify each parent and whether they were in the same social group at conception. We used the pedigree constructed in (Wells et al., 2018) and the full details of construction are described therein and in (Sanderson et al., 2015). In brief, parentage was principally assigned using MasterBayes (Hadfield, Richardson, & Burke, 2006) because it can incorporate phenotypic and genetic data when calculating assignment probability. This approach was also supplemented by using Colony (Jones & Wang, 2010) to identify sibship groups which allowed us to identify related founders or immigrants rather than assuming them to be unrelated. The cut-off probability for accepting parentage assignments was 0.8 but in actuality the average confidence of the accepted MasterBayes assignments was 0.99. The same cut-off probability was used for Colony assignments but they were only accepted if MasterBayes failed to confidently assign parentage. The final pedigree was 9 generations deep and included 1,547 individuals with both parents confidently assigned.

Genetic and life history variables

Once parentage was assigned we could identify whether pups were sired by within-group or extra-group males. Extra-group paternity was identified when the mother was not in the same group as the father during the window of conception, specifically the second and third months prior to giving birth. Females give birth to up to six pups at a time. Here the pups of a single female from a single breeding event are referred to as a “brood” (see Table 1). As previously noted, multiple females in a group may give birth during a single breeding event and all of these offspring are collectively referred to as a “litter” which may contain multiple broods. Over 90% of broods resulted solely from within-group or extra-group mating despite mixed paternity in some broods; this indicates that the extra-group sire status of each pup is not independent of the rest of the brood’s status, presumably because it is possible for a female’s entire brood to be sired during a single mating. Therefore, each brood was assigned a binary extra-group paternity status, 1 if any pup’s father was extra-group and 0 if no pup in the brood had an extra-group father.

Table 1. Definitions of terms used throughout the paper for quick reference.

Term	Definition
Breeding event	Breeding is highly synchronised within groups to breeding events.
Brood	The pups born to a single female in a single breeding event.
Litter	Collective term for all pups in a group born during a single breeding event.
f_b	The average inbreeding coefficient of a brood.
Group age	Time between brood conception and the first time the group was observed.
Relatedness to top males	The average pedigree relatedness of a focal female to males of the top three age ranks (assuming they are over 6 months old) and immigrants to the population.
Group size	The number of individuals over six months old in the mother's group at conception.
In natal group	Females still in the group they were born in when they conceived were said to be in natal group.
Age	Age of the mother at conception.
Extra-group breeding	Pup was fathered by a male which was not in the mother's social group in either the second or third month before birth.
Cohort	Adults born in the same litter are referred to as a cohort and are very similar ages.

The pedigree was also used to calculate the pups' inbreeding coefficient and the average relatedness of mothers to top ranked males. Individual inbreeding coefficients (f) were calculated using the inverseA function from the MCMClgmm R package (Hadfield, 2010). The average inbreeding coefficient of each brood (f_b) was calculated as the average f of all pups in the brood which had both parents confidently assigned and present in known groups at conception. Nichols et al. (2015) found that the occurrence of extra-group paternity was not associated with the average relatedness between members of the opposite sex within a group. However, in the banded mongoose 85% of within-group reproduction is monopolised by males of the top three age ranks (Nichols et al., 2010). A male's age rank was calculated as the number of older males in the group +1; males born in the same litter were all given the same rank. We calculated the average relatedness between a focal female and males with rank 1-3 in her social group (assuming they were over six months old). Male immigrants to the study site of unknown age were also included when calculating relatedness as they were expected to be unrelated and therefore represent an opportunity for outbreeding despite breeding within-group.

Each group was visited every 1-4 days to record group and life history variables. Females give birth in an underground den so could not be directly observed but we can infer the date of birth from a visible reduction in the size of the mother's abdomen as groups with pregnant females were visited every day. From this we estimated the date of conception by assuming a gestation period of 60 days (Cant, 2000). The age of the mother at conception was recorded in years. Group age at brood conception was recorded as the number of years since the social group was first observed. Group size was measured as the number of individuals over six months old in the mother's social group at conception.

Statistical analysis

We were interested in 1) how social groups change, 2) how this affects female extra- vs within-group breeding, and 3) how the inbreeding coefficient of resulting offspring was affected by this breeding behaviour. As many aspects of banded mongoose breeding behaviour have been studied over the last 23 years, we were able to propose which variables should be related and the direction of causality based on prior knowledge. This was laid out as a series of paths creating a piecewise structural equation model. Based on knowledge of the banded mongoose breeding system, group size, group relatedness, and extra-group breeding should all be dependent on group age (Cant et al., 2016; Nichols et al., 2015, 2012). Females in their natal group should differ in both their relatedness to top males and their extra-group breeding behaviour compared to females not in their natal group (Nichols et al., 2014). In addition to group age and being in her natal group, female extra-group breeding should depend on her group's size as it influences the outcome of inter-group interactions (Thompson, Marshall, Vitikainen, & Cant, 2017). In order to investigate how mating behaviour changes over a female's life we included her age. Finally, f_b should depend on the relatedness of the mother to the top ranked males in her group but only for within-group breeding (Nichols et al., 2010). These paths are more succinctly described as equations in Table 2.

Table 2. The fixed effect equations composing the structural equation model. Written in the R mini-language

Group size ~ group age
Relatedness to top males ~ group age + mother in natal group
Extra-group breeding + mother in natal group + group size + age
Average brood inbreeding coefficient ~ mother in natal group * relatedness to top males

The equations in Table 2 were fit as GLMMs in R (R Core Team, 2018) and combined into a piecewise structural equation model using the R package piecewiseSEM (Lefcheck, 2016). For all models our dataset was 662 broods born to 108 females in 271 breeding events across 16 social groups. The models are described in detail below but all included mother, litter, and mother's group at conception as random effects. All continuous variables were mean centred and standardised by their standard deviation so that effect sizes could be compared across the model (Schielzeth, 2010). Relationships which are not specified in our models are believed to be biologically unimportant. These unspecified relationships are called independence claims and we test that the variables in them are independent after accounting for the relationships which are specified. These tests compare the originally specified path with one including the independent variable (see supplementary material SM3). If the independence claim is non-significant it indicates that the variables are conditionally independent. The *p*-values of these independence claims are then used collectively to calculate *Fisher's C* statistic to test whether the structural equation model captures the relationships in the data.

1) Societal changes

The first two models evaluated how social groups change through time. Group size and relatedness to top males were fitted as response variables in separate linear mixed models using the R package lme4 (Bates, Maechler, Bolker, & Walker, 2015). Group age was fit as a fixed effect in both models because new groups get larger over time (Thompson, Marshall, Vitikainen, Young, & Cant, 2017) and due to limited dispersal, relatedness also increases over time (Nichols et al., 2012). Whether the mother was in her natal group or not was included as a fixed effect in the model of relatedness because in a female's natal group, her father and other male relatives are potential mates. In the model of relatedness, group age was fit as a random slope by group identity; this was based on data inspection and supported by AIC.

2) Extra- or within-group breeding

Next we investigated how social variables influenced female within- or extra-group breeding. Extra-group paternity was fit as a binary response for each brood in a binomial GLMM in the R package lme4 (Bates et al., 2015). The age of the group was included as a continuous fixed effect while whether or a not a female conceived in her natal group was included as a binary fixed effect because both of these variables have been previously associated with extra-group breeding in the banded mongoose (Nichols et al., 2014, 2015). Group size was also included in the model as females in larger groups will have a wider selection of potential mates for within-group breeding and this species does express some preference for less related mates when breeding within-group (Sanderson et al., 2015). The breeding female's age at conception was also included as a fixed effect in order to investigate how female breeding behaviour changes over time.

The individual and other random effect repeatabilities were calculated from the random effect variance estimates. Following (Nakagawa & Schielzeth, 2010) the random effect variances were converted into repeatability for a logit distribution with additive overdispersion as

$$R_{logitA} = \frac{\sigma_x^2}{\sigma_{mother}^2 + \sigma_{litter}^2 + \sigma_{group}^2 + \sigma_e^2 + \frac{\pi^2}{3}}$$

Where σ_{mother}^2, σ_{litter}^2 and σ_{group}^2 are the random effect variances of the mother, litter and social group random effects respectively. The random effect variance to be converted into a repeatability is indicated by σ_x^2 and the residual variance is σ_e^2. Note that as our response variable was binary, σ_e^2 was inestimable (Nakagawa & Schielzeth, 2010) and was set to 1.

3) Inbreeding coefficient of resulting offspring

The final model in our piecewise structural equation model focused on how inbred the resulting brood of offspring were. The mean inbreeding coefficient of the brood (f_b) was fit as the response term in a linear mixed model using the R package lme4 (Bates et al., 2015). As fixed effects, we fit the average relatedness of the breeding female to top ranked males, whether or not any pups in the brood were sired by extra-group males (binary extra-group breeding status), and an interaction between these two terms. The interaction was included because the relatedness between within-group individuals is only relevant to f_b for within-group breeding.

The significance of fixed effects in the mixed models was assessed either through parametric bootstrapping or using Markov chain Monte Carlo (MCMC) methods. Parametric bootstrapping was performed using the pbkrtest package in R (Halekoh & Højsgaard, 2014). A reduced model was created for each fixed effect by dropping that variable from the model and data was simulated according to this simplified model 1000 times. The full and simplified models were compared using likelihood ratios for all 1000 simulated datasets. The *p*-value was calculated as the number of simulated likelihood ratios which were greater than or equal to the observed likelihood ratio. Due to convergence issues in the reduced models, but not the full model, parametric bootstrapping was not appropriate for our model of extra-group breeding. Instead, the model was refit using MCMCglmm (Hadfield, 2010) as described in the supplementary material, SM1, because accurate *p*-values can be calculated from the posterior distribution. All models were validated by checking histograms of residuals and plots of residuals against predictors for trends. Colinearity was evaluated by calculating the variance inflation factor which was below 2 for all models.

Results

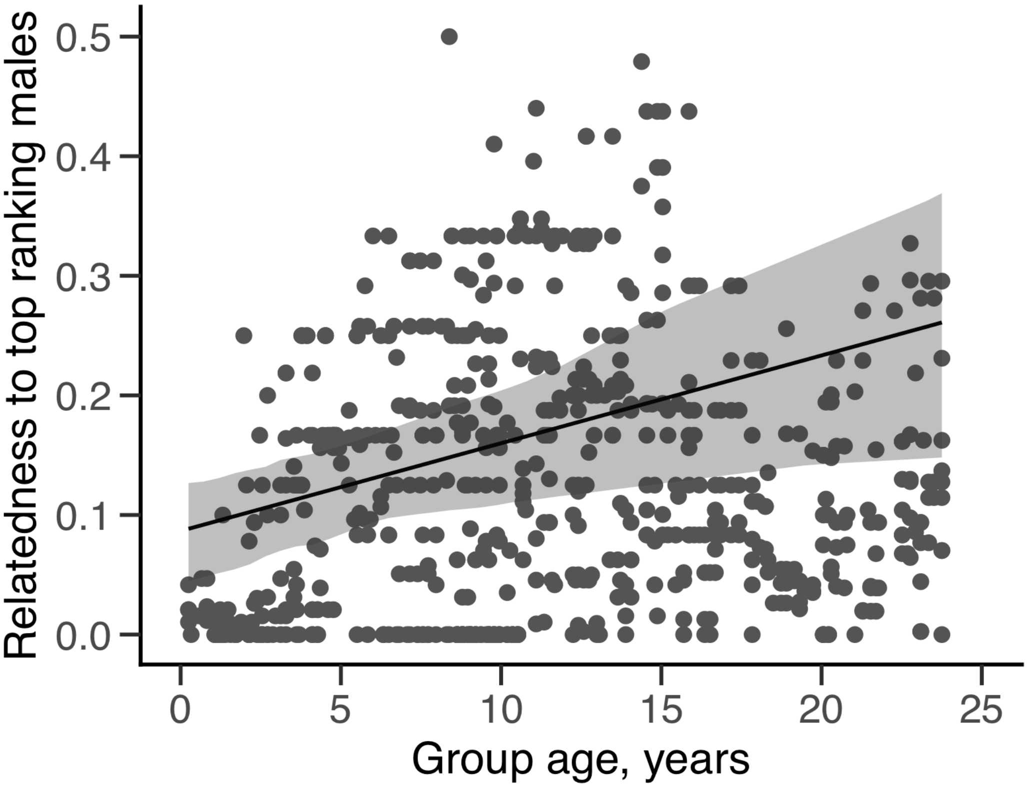
1) Societal changes

We found that both group size and within-group relatedness increased with group age. Group size was significantly correlated with group age (group age $ß = 0.15$, $p = 0.034$) which is expected because newly formed groups are usually smaller than more established groups (Thompson, Marshall, Vitikainen, Young, et al., 2017) but become larger over time as new offspring are born and mature. The relatedness of breeding females to the top-ranking males also increases over time (group age $ß = 0.41$, $p < 0.001$, Figure 1). Relatedness is initially very low as the opposite sex founders are from separate groups and so are unrelated. However, relatedness increases over time because most breeding occurs within group and the founders' offspring commonly remain in their natal group until they too become breeders (predicted relatedness of philopatric mothers to top males was 0.16 after 10 years). Although some females disperse away from their groups to become group founders, the majority (>80%) remain in their natal group for their whole lives (Cant et al., 2016). The relatedness between breeding females and top-ranking males is much higher if the female is in her natal group (mother in natal group $ß = 0.77$, $p = 0.004$) because the top-ranked males are likely to include her father and other male relatives. See Table 3 for full model outputs.

Table 3. Reports the fixed effects and their significance from the models which make up our piecewise structural equation model. The significance of the fixed effects was determined either by parametric bootstrapping (PB) or Markov chain Monte Carlo methods (MCMC).

Response	Fixed effect	Estimate	Std. Error	*p*-value	Method
Group size	(Intercept)	-0.69	0.22		
	Group age	0.15	0.07	0.034	PB
Relatedness	(Intercept)	-0.41	0.31		
	Group age	0.41	0.18	0.001	PB
	Mother in natal group	0.77	0.23	0.004	PB
Extra-group Paternity	(Intercept)	-6.67	0.05	0.001	MCMC
	Group age	0.77	0.01	0.040	MCMC
	Mother in natal group	3.42	0.04	0.004	MCMC
	Group size	-0.95	0.01	0.004	MCMC
	Age	0.9	0.01	0.001	MCMC
Brood inbreeding coefficient	(Intercept)	0.15	0.05		
	Extra-group breeding	-0.61	0.08		
	Relatedness	0.55	0.04		
	Interaction	-0.49	0.08	0.001	PB

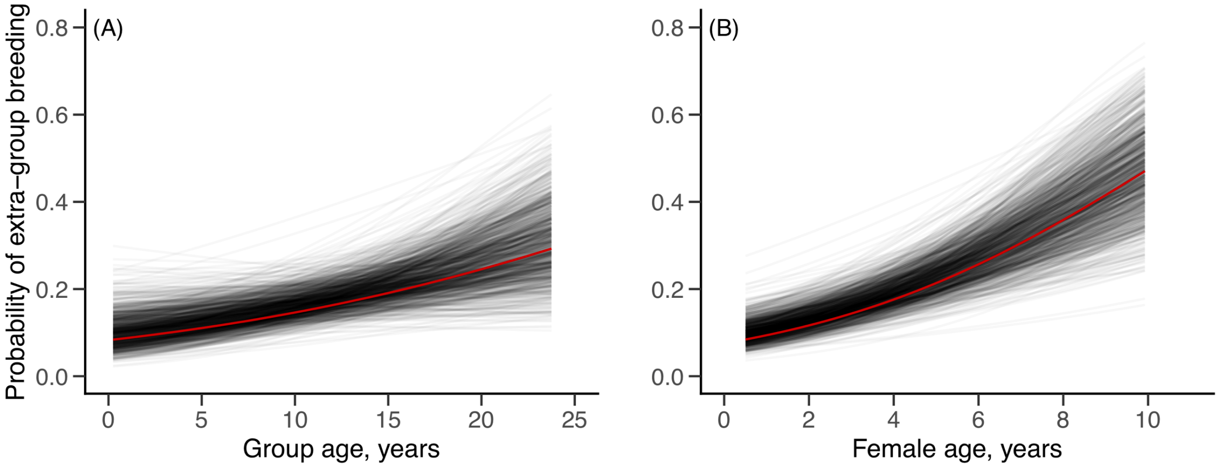
Figure 1. Plot shows the relatedness of breeding females to the top ranked males in her social group as a function of the group's age. The trend line shows the fitted model for females in their natal group and the shaded region shows the 95% CI.



2) Extra- or within-group breeding

Females display adaptive plasticity in their breeding strategy such that extra-group breeding is more common when the risk of inbreeding is greater. Females were more likely to engage in extra-group breeding if they were in their natal group (mother in natal group $ß = 3.42$, $p = 0.004$) and when their group was older (group age $ß = 0.77$, $p = 0.040$, Figure 2A). As discussed above, both of these variables are associated with increased relatedness and so should be reasonable proxies for the risk of inbreeding. However, relatedness between females and top-ranking males was not directly correlated with a female's probability of extra-group breeding after accounting for other fixed effects as shown by the non-significant independence claim (relatedness $ß = 0.30$, $p = 0.099$). Females in larger groups were less likely to engage in extra-group breeding (group size $ß = -0.95$, $p = 0.004$) whereas older females were more likely to breed with extra-group males (age $ß = 0.9$, $p < 0.001$, Figure 2B). See Table 3 for full model outputs.

Figure 2. Plots show the probability of females breeding with extra-group males as a function of (A) age of the social group and (B) age of the female. The red trend lines show the fitted model based on the posterior mean of all coefficients for females in their natal group with all explanatory variables fixed to their average except for that displayed on the x-axis. Each pale grey line represents the fitted model based on a single draw from the posterior distribution of fixed effect coefficients.

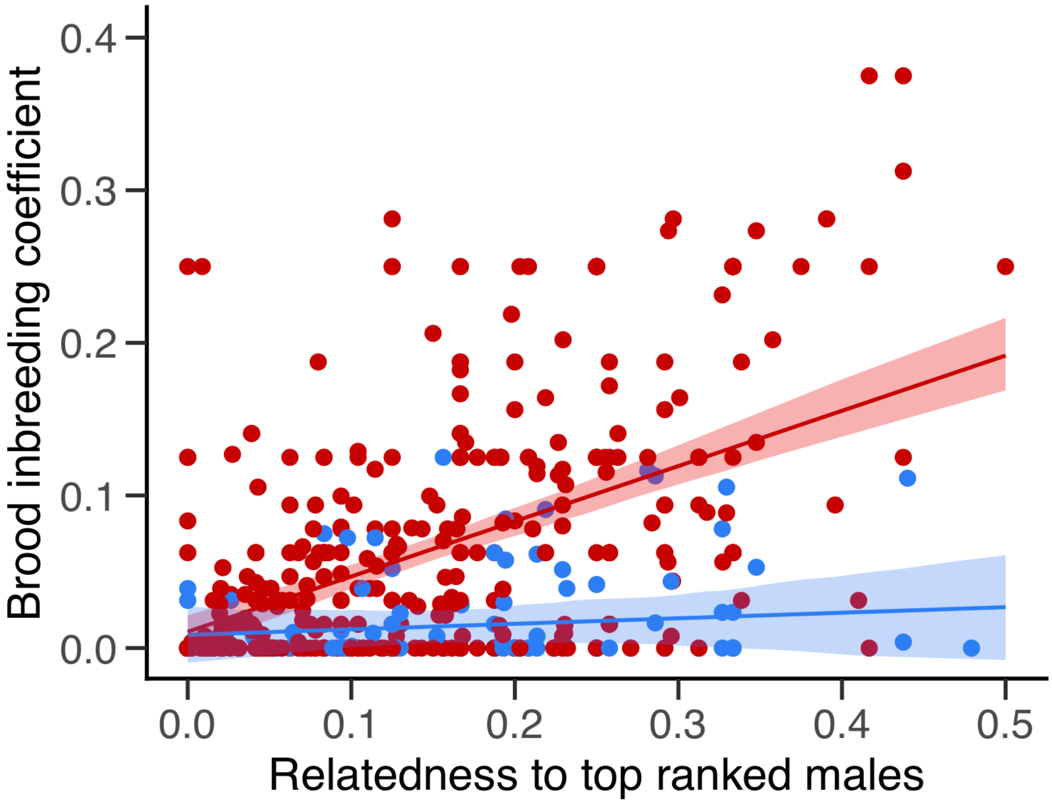


Despite plasticity in breeding behaviour, there was some evidence that females also displayed individual repeatability. The posterior mode of individual repeatability was 0.09 indicating that almost 10% of the variance in extra- vs within-group breeding could be explained by female identity, after fixed effects had been accounted for. Although the mode of this posterior distribution was located away from zero, this is not unequivocal evidence of individual repeatability as the 95% CI overlapped zero (see the supplementary material for details, SM1). The equivalent repeatability for litter was 0.50 indicating that females breeding in the same breeding event behaved similarly. In contrast, females in the same social group were not alike more generally as the social group repeatability was zero.

3) Inbreeding coefficient of resulting offspring

As expected, the relatedness between a female and the top ranked males in her group was correlated with the inbreeding coefficient of her offspring (Figure 3). However, breeding with extra-group mates successfully avoids inbreeding as shown by the significant interaction between relatedness and extra-group breeding (relatedness *ß* = 0.55, extra-group breeding *ß* = -0.61, interaction *ß* = -0.49, p <0.001, Figure 3). See Table 3 for full model outputs. The distribution of f_b violates the assumption of normality as it is bounded at zero; however, analyses using sMLH to measure inbreeding were qualitatively similar which indicates that our results are robust to this violation (see supplementary material SM2). Despite high levels of inbreeding among within-group sired pups, only 18% of broods contained at least one pup with an extra-group father.

Figure 3. Plot shows the average inbreeding coefficient of a brood as a function of the mother's relatedness to the top ranked males in her social group and whether the brood resulted from within-group breeding (dark red) or extra-group breeding (light blue). Trend lines show the fitted model and the shaded regions show the 95% CI.



Combining our models into a single structural equation model

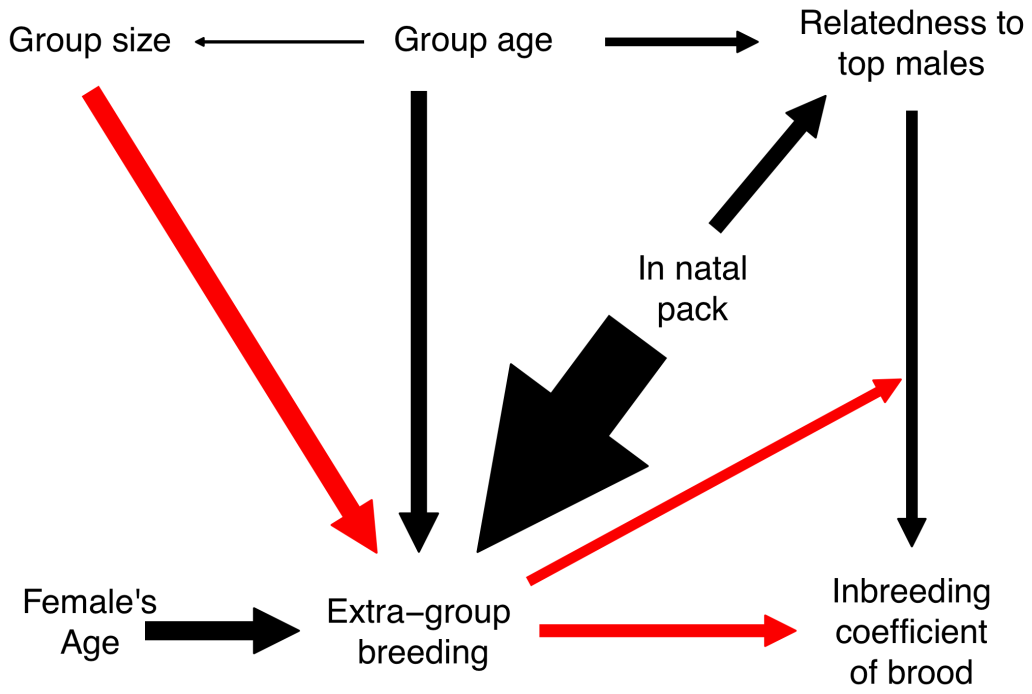
As described above, relatedness to top-males and group size change over time which affects the breeding decisions of females and ultimately the f_b of the resulting brood. All of the fixed effects specified in Table 2 were significant (see Table 3) and these relationships are visualised in Figure 4. Combing our models into a piecewise structural equation model shows that there are no other important relationships in our dataset as confirmed by the non-significant independence claims (p>0.05, Table S1 in supplementary material SM3). The p-values of these independence claims were used to calculate a global goodness-of-fit measure which also indicated all of the important relationships in our dataset were included in our structural equation model

(*Fisher's* $C = 22.38$, $df = 18$, $p = 0.215$) and therefore that our specified relationships accurately represent the relationships observed in our data (Lefcheck, 2016). The models which make up our piecewise structural equation model all have considerable explanatory power as shown by high R^2 values (Table 4), especially conditional R^2 which includes the explanatory power of random effects. The conditional R^2 is very high (~1) for our model of group size because females within groups give birth on the same night and so the group size is identical for all females contributing to a given litter. The conditional R^2 for female relatedness to top ranking males is also very high (0.92) because a females' relatedness to the oldest males only changes when one of them dies or she changes social group.

Table 4. Marginal and conditional R^2 values describe the proportion of variance in the response term explained by the fixed or fixed and random effects respectively. Family is the chosen distribution underlying the model and link describes the chosen link function. For the binomial model R2 values were calculated using the theoretical variance associated with the link function.

Response	Family	Link	Marginal R^2	Conditional R^2
Group size	Gaussian	Identity	0.02	1.00
Relatedness to top ranked males	Gaussian	Identity	0.17	0.92
Extra-group paternity	Binomial	Logit	0.14	0.65
Brood inbreeding coefficient	Gaussian	Identity	0.32	0.49

Figure 4. Path diagram represents the fitted piecewise structural equation model. Arrows represent the relationship between variables, black is a positive effect and red is a negative effect. The width of the arrow is proportional to the magnitude of the coefficient. Arrows directed to other arrows represent interactions.



Discussion

We find that inbreeding avoidance through extra-group mating is a dynamic process, being influenced by a complex web of social, demographic, and individual factors. Female banded mongooses adaptively breed with extra-group males when the risk of inbreeding within their group is high. However, female extra-group breeding behaviour was not directly influenced by her relatedness to within-group males but varied with two proxies for relatedness, group age and whether she was in her natal group or not, as well as two other variables; namely her own age and the size of her group. Despite plastic variation in breeding behaviour, only 18% of all broods were at least partially sired by extra-group males. High within-group relatedness meant that 20% of within-group broods were at least moderately inbred ($f_b \geq 0.125$), whereas extra-group offspring were consistently outbred. This frequency of inbreeding likely indicates that breeding behaviour was shaped in part by selection pressures other than inbreeding depression.

Extra-group breeding was not based on relatedness directly but instead on proxies for relatedness. Female behaviour was affected by two proxies for within-group relatedness; these proxies were group age and whether the female was in her natal group or not. Proxies such as these are commonly used as cues for phenotypic plasticity but environmental change can lead to cue-environment mismatch and low fitness (Bonamour et al., 2019). For example, because the relationship between air temperature and snow melt has changed there is significantly more snow on the ground when yellow bellied marmots emerge from hibernation; as a result the marmots suffer a reduction in foraging opportunities (Inouye, Barr, Armitage, & Inouye, 2000). Similarly, banded mongoose fitness could be affected in the future if the relationship between group age and within-group relatedness changes.

Despite behavioural plasticity, we uncovered a non-significant trend for females to show individual repeatability in their tendency to breed with extra-group males. This means that despite varying their behaviour with prevailing environmental conditions they were consistently more or less likely to engage in extra-group breeding than the population average expectation for those conditions. Our individual repeatability estimate (0.09) is slightly lower than estimated in the Mandarte island song sparrow for the equivalent behaviour (0.19) (Reid, Arcese, Sardell, & Keller, 2011) but it is much lower than the female repeatability of the proportion of extra-pair young in tree swallows (0.83) (Whittingham, Dunn, & Stapleton, 2006). The high repeatability in tree swallows could be because broods were compared within a single breeding season whereas in our study and in the song sparrow broods were compared across breeding seasons over several years. Individual consistency may be an important consideration for conservation as individuals which will reliably attempt to outbreed can be selected. Alternatively, individuals which do not refuse relatives as mates could be easier to manage in breeding programmes when the available pool of breeders is limited as is common in captivity.

Individual repeatability sets an upper limit on heritability (but see Dohm, 2002) and so behaviours must be repeatable in order to respond to selection. This repeatability must stem, at least partly, from additive genetic variance rather than entirely from permanent environmental effects in order to be heritable (Falconer & Mackay, 1996; Lynch & Walsh, 1998). Very few studies have estimated additive genetic variance in breeding behaviour, in part because it requires such large data sets. We used simulated data to demonstrate that our dataset (662 broods born to 108 females) did not have the statistical power necessary to separate additive genetic variance and permanent environmental effects (see supplementary material SM4). Reid et al. (2011) found that almost all within individual repeatability in female extra-group breeding could be attributed to additive genetic variation. Even if the genetic architecture is similar in the banded mongoose our estimate of individual repeatability would still indicate a relatively low rate of evolution in extra-group breeding behaviour.

Inbreeding depression is generally expected to select for inbreeding avoidance but the banded mongoose frequently inbreeds (Nichols et al., 2014; Wells et al., 2018) despite suffering inbreeding depression (Mitchell et al., 2017; Sanderson et al., 2015; Wells et al., 2018). Inbreeding depression is not the only selection pressure shaping the evolution of breeding behaviour; the cost of inbreeding avoidance behaviours will oppose their evolution (Duthie & Reid, 2016; Kokko & Ots, 2006). For example, breeding with relatives was actually selected for in female Mandarte island song sparrows, despite inbreeding depression (Reid et al., 2015). This positive selection was partly explained by females with related mates raising more offspring per season, which implies some cost to seeking unrelated mates.

In the banded mongoose extra-group breeding appears to be very costly as it occurs during aggressive interactions between groups. On sighting a rival group, individuals will give a distinctive call and bunch together. If one group is much smaller that group will often retreat, but groups will fight aggressively if they are evenly matched (Thompson, Marshall, Vitikainen, & Cant, 2017). Pups and adults are often injured during these fights and 15% of deaths with a known cause can be attributed to these fights (Nichols et al., 2015). These inter-group interactions are therefore costly to a female because of the risk to herself, her offspring, and her group members. Even if the focal female or her relatives are not harmed, a reduction in group size can negatively impact territory size and survival (Kokko, Johnstone, & Clutton-Brock, 2001). Despite violence, extra-group breeding is believed to occur during these inter-group interactions as females have been observed mating with extra-group males during them (Nichols et al., 2015). Inter-group interactions are more common when females are in oestrus (Thompson, Marshall, Vitikainen, & Cant, 2017) and females are more likely to conceive to extra-group males after an inter-group interaction (Nichols et al., 2015), further implicating these interactions as an opportunity for extra-group breeding. Within- vs extra-group breeding therefore likely represents a trade-off between the risk of inbreeding and the risk of violence.

Reductions in the risk of violence would be expected to lead to an increase in extra-group breeding. Older females engage in more extra-group breeding, and this would be explained if older females suffer lower costs from inter-group interactions. In keeping with this hypothesis, a higher proportion of pup deaths are related to intergroup interactions than adult deaths (Nichols et al., 2015). However, unpublished results suggest that, in contrast to males, females over one year old are rarely killed during intergroup interactions (Faye Thompson, personal communication). This suggests that the risk of violence cannot decrease greatly with age beyond one year in females. An alternative explanation for the relationship between female age and extra-group breeding is that females use their own age as a proxy for relatedness to top males. Although a female may not be able to measure their group's age directly, it will increase over her lifetime and females in older groups are more related to top males.

Females in larger groups were less likely to have bred with extra-group males, although it is not immediately clear why. Females in large groups have more within-group males to choose from and so may be able to avoid inbreeding without resorting to extra-group breeding. However, group size does not correlate with the inbreeding coefficient of broods fathered by within-group males which we would expect if females in larger groups could successfully avoid within-group inbreeding (group size *ß = 0.08, p* = 0.089, see SM5). Alternatively, this effect of group size may indicate a change in the way groups interact. Larger groups are more likely to win inter-group conflicts, but if anything that should reduce the cost (Cant, Otali, & Mwanguhya, 2002; Thompson, Marshall, Vitikainen, & Cant, 2017) and lead to more extra-group breeding. However, when there is a large asymmetry in group size the smaller group often flees rather than fights (Thompson, Marshall, Vitikainen, & Cant, 2017), potentially limiting the number of opportunities for extra-group breeding. Another possibility is that group size is being used as a proxy for relatedness to top males, as groups become larger and relatedness to top males increases over time.

Identifying factors which shape an individual's breeding decision will also help to identify the factors which shape the evolution of breeding behaviour across species. Within-group relatedness has been reported to encourage extra-group breeding in other species too (Annavi et al., 2014; Cohas, Yoccoz, Da Silva, Goossens, & Allainé, 2006). These similar results imply that likelihood of encountering relatives is an important determinant of inbreeding behaviour as expected by theory (Jamieson et al., 2009). The cost of inbreeding avoidance also appears to have shaped the evolution of breeding behaviour in the banded mongoose, but it is less clear how important theses costs are in general. Although the cost of inbreeding depression has been studied in many species (Crnokrak & Roff, 1999; Keller & Waller, 2002) the costs of inbreeding avoidance have been estimated less often (Forstmeier, Nakagawa, Griffith, & Kempenaers, 2014) despite theoretical results implying that they should be important in determining the evolution of inbreeding behaviour (Duthie & Reid, 2016; Kokko & Ots, 2006).

Different types of inbreeding avoidance behaviour may have costs of differing magnitude. For illustration, extra-pair breeding may reduce a clutch's fitness by 2% on average due to reduced investment by the social mate. This estimate is based on comparative data and assumes: an 11% rate of extra-pair breeding (Griffith, Owens, & Thuman, 2008), that complete extra-pair breeding reduces male care by 43% (Arnqvist & Kirkpatrick, 2005), and that complete removal of paternal care reduces clutch fitness by 49% (Møller, 2000). By contrast, delayed reproduction may strongly reduce fitness if breeding success is zero for several seasons; for example, lifetime reproductive success is reduced 20% by delaying breeding for one year in the yellow bellied marmot (Armitage & Downhower, 1974; Van Vuren & Armitage, 1994). If different types of inbreeding avoidance behaviour do differ in their costs it yields a testable prediction; species which employ costly forms of inbreeding avoidance should tolerate higher levels of inbreeding. However, comparable estimates of inbreeding avoidance costs in various species will be needed to assess their general importance in the evolution of inbreeding avoidance, tolerance and preference.

Conclusion

We have shown that the risk of inbreeding changes over time and that females adaptively adjust their breeding behaviour in accordance with this risk. However, they do not measure the risk of inbreeding directly, instead changing their behaviour according to proxies which are ostensibly more easily measured. Despite these adaptive behavioural changes 82% of broods had within-group sires and of those broods 20% were at least moderately inbred ($f_b \geq 0.125$) which implies severe costs to this inbreeding avoidance strategy. The high cost of inbreeding avoidance may be the principal driver of frequent incest in this species; however, it is unclear if this is a general result as estimates of these costs are rare and difficult to obtain for most behaviours.

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Supplementary material

SM1 MCMC model of extra-group breeding

This section describes how we refit the extra-group breeding model, described in the main text and Table 3, using MCMCglmm (Hadfield, 2010). Refitting the model had two advantages; we could accurately assess the significance of the fixed effects and come to a more complete understanding of the estimated variance components, most interestingly individual repeatability and litter repeatability. Both of these advantages are inherent in any statistical framework that estimates the full posterior distribution of the model parameters.

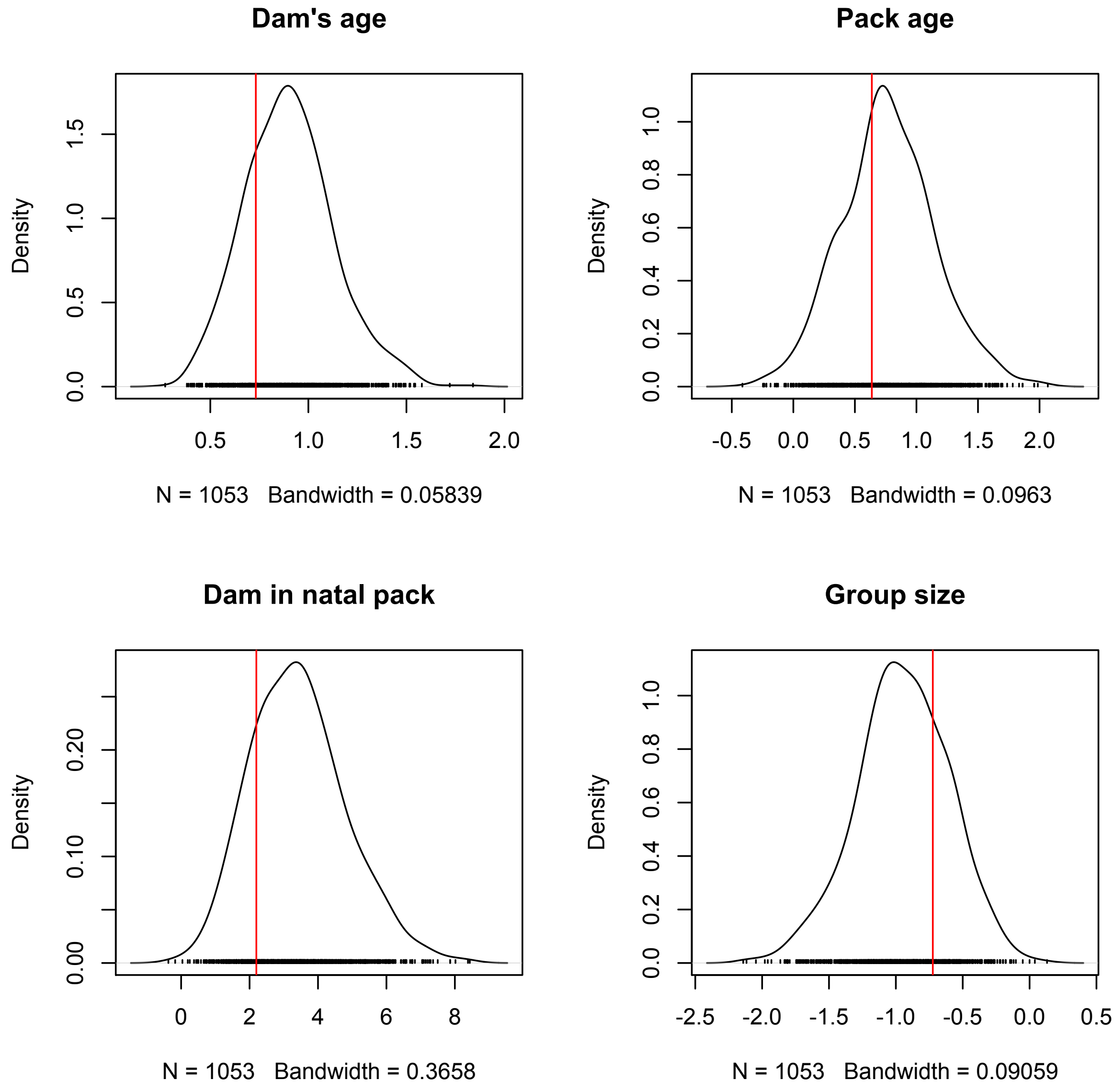
The significance of fixed effects in the extra-group breeding model could not be determined initially due to convergence errors. In other models, the significance of fixed effects was determined through parametric bootstrapping as described in the main text (see Table 3) and implemented using the R package pbkrtest (Halekoh & Højsgaard, 2014). However, when bootstrapping the extra-group breeding model a large proportion of the models fit to simulated data failed to converge, and so the significance could not be accurately determined.

The extra-group breeding model was refit using MCMCglmm using the same fixed and random effect structure. Parameter expanded priors (V=1, nu=0.002, alpha.mu=0, alpha.V=1000) were used for each random effect and the residual variance was fixed to one as it is inestimable for binary models (Nakagawa & Schielzeth, 2010). Because the residual variance was fixed, the random effect variances can only be meaningfully interpreted after rescaling, which was done following (Nakagawa & Schielzeth, 2010) for a binomial model with a logit link and additive overdispersion, as described in the main text.

The model was run for 5,265,000 iterations with a burn-in of 3000 and a thinning interval of 5000 iterations. Trace plots and Geweke plots were checked for each parameter to ensure good mixing of the chain and convergence. Autocorrelation and effective sample size were also checked to ensure sufficient sampling of the posterior distribution.

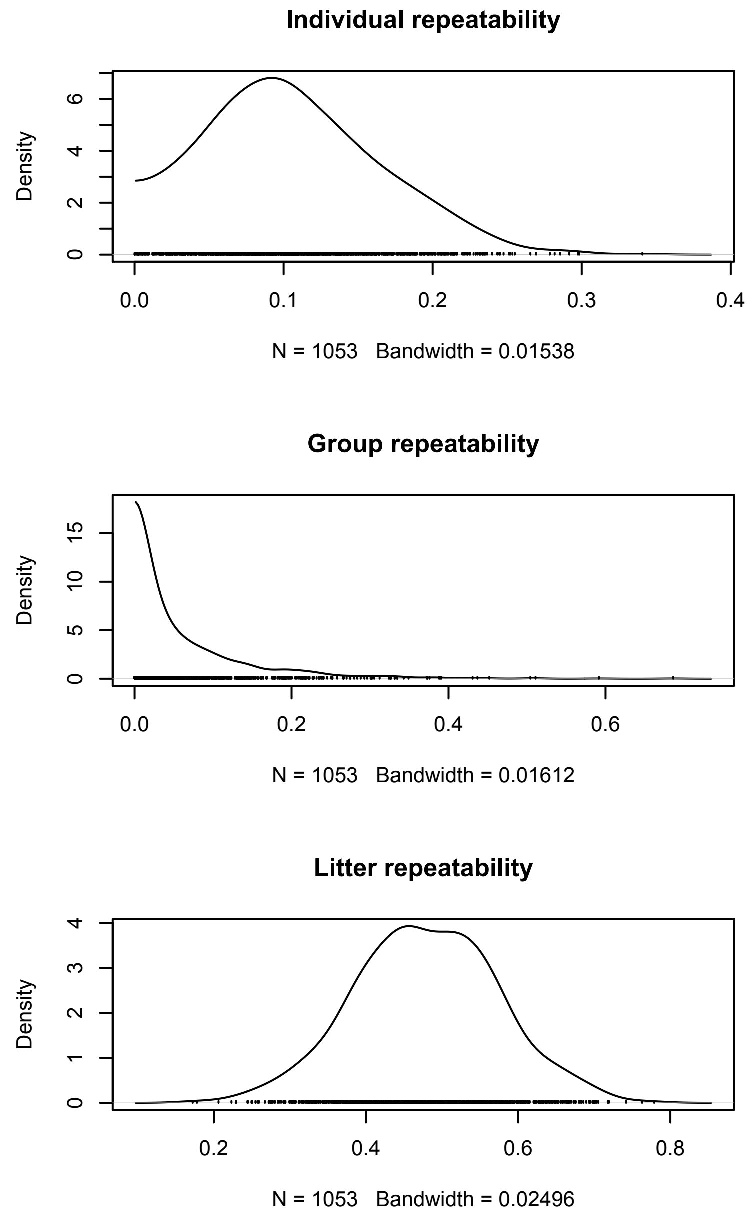
The fixed effect coefficients estimated by glmer and MCMCglmm showed good agreement (Figure S1). This agreement indicates that both models successfully converged on the correct set of model parameters.

Figure S1. Density plots showing the posterior distribution of the fixed effect coefficients from the MCMCglmm extra-group breeding model. The red vertical line indicates the point estimate from the model fit using lme4. The ticks along the x-axis indicate individual draws from the posterior distribution. The text below each panel indicates the number of draws from the posterior distribution and the bandwidth used when calculating the density.



The posterior distribution of the individual repeatability is asymmetrical and so difficult to describe using traditional point estimates and standard deviations. This illustrates one of the advantages of describing the complete posterior distribution of variance components as they are often asymmetrical. The posterior mode of individual repeatability is 0.09 but the 95% posterior density intervals overlaps zero as shown in Figure S2 (0.02-0.21). It is therefore somewhat unclear whether females are repeatable in their extra-group breeding tendencies. In contrast, females in the same social groups are clearly not consistent (posterior mode = 0.00, 95% posterior density interval = 0.00-0.23, Figure S2), whereas females giving birth to pups in the same litter do show significant repeatability (Figure S2, posterior mode = 0.50, 95% posterior density interval = 0.30-0.67). See the discussion in the main text for further details.

Figure S2. Density plots showing the posterior distribution of random effect repeatability for the MCMCglmm extra-group breeding model. The ticks along the x-axis indicate individual draws from the posterior distribution. The text below each panel indicates the number of draws from the posterior distribution and the bandwidth used when calculating the density.



SM2 Predicting offspring heterozygosity

In the main paper we focused on pedigree inbreeding coefficient to quantify inbreeding as it is directly interpretable (0.25 is equivalent to breeding between full siblings) and generally explains more variation in fitness compared to heterozygosity estimates (Balloux, Amos, & Coulson, 2004; Nietlisbach et al., 2017; Slate et al., 2004). However, validation of our model predicting the average brood inbreeding coefficient showed evidence of heteroscedacity, specifically an increase in residual variance with increasing relatedness values. This is likely because inbreeding coefficient does not follow a normal distribution but has a lower bound of zero. Here we fit an equivalent model using average brood sMLH to estimate inbreeding and demonstrate that our results are not dependant on our measure of inbreeding.

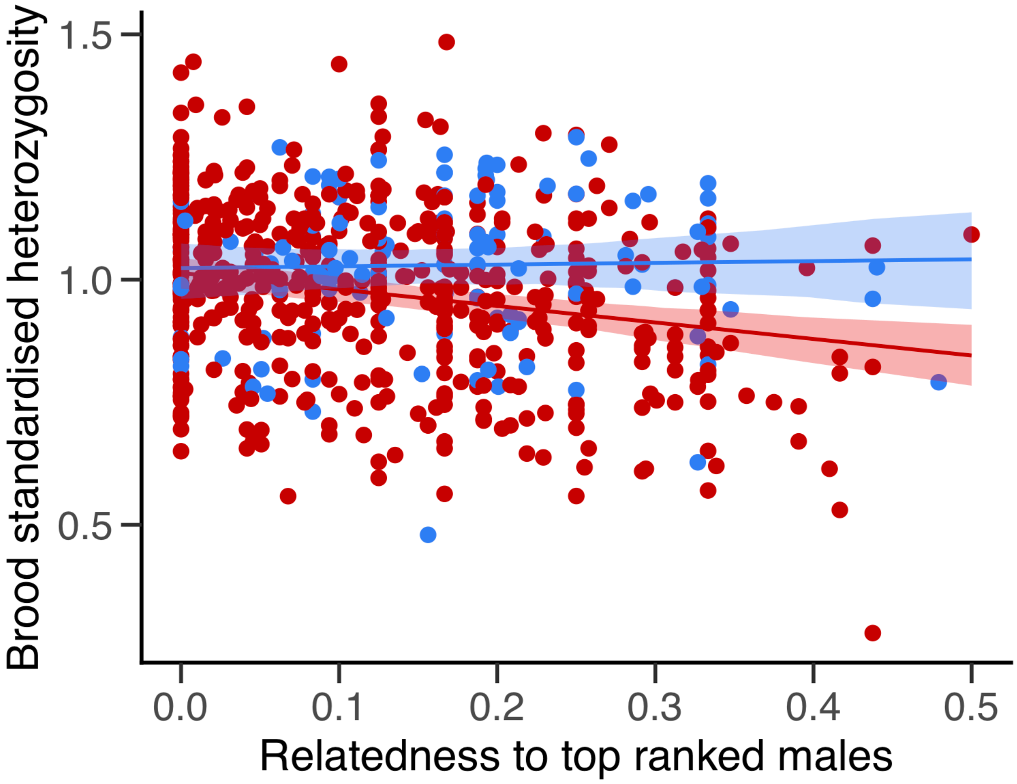
We investigated the effect of extra-group breeding and relatedness to the top ranked males on the average brood heterozygosity. Heterozygosity was calculated as standardised multi-locus heterozygosity (sMLH) using the R package inbreedR (Stoffel et al., 2016). The model was fit as a LMM in the R package lme4 (Bates et al., 2015). As in the model using inbreeding coefficients, we included an interaction between relatedness and the extra-group paternity status of the brood because the relatedness between within-group individuals should not influence extra-group inbreeding. The random effect structure was also the same and included a random intercept for mother, mother's social group at conception and litter.

The significance of the interaction term was assessed through parametric bootstrapping. A reduced model was created by dropping the interaction term then both the reduced and full model were fit to the observed data to generate an observed likelihood statistic. The significance of this statistic was assessed by comparison to a distribution of similarly calculated likelihood statistics based on 1000 simulated datasets generated according to the reduced model. This procedure was conducted using the R package pbkrtest (Halekoh & Højsgaard, 2014).

Using sMLH as a measure of inbreeding did not qualitatively change our results compared to using pedigree inbreeding coefficients. As a female became more related to the top ranked males in her group, the expected heterozygosity of her within-group offspring decreased; this is equivalent to an increase in inbreeding (see Figure S3). By contrast, extra-group brood sMLH was independent of relatedness, which was shown by a significant interaction coefficient approximately equal and opposite to the relatedness coefficient (relatedness *ß* = -0.22, extra-group breeding *ß* = 0.35, interaction *ß* = 0.25, p = 0.009).

Average brood sMLH is normally distributed and visual inspection of residual plots confirmed that the assumptions of this model were not violated. This supports the conclusion of our model based on inbreeding coefficients and implies that those results are not due to heteroscedasticity in the model.

Figure S3. Plot to show the average heterozygosity (sMLH) of broods as a function of the mother's relatedness to the top ranked males in her social group and whether the brood resulted from within-group breeding (dark red) or extra-group breeding (light blue). Trend lines show the fitted model and the shaded regions show the 95% CI.



SM3 Independence claims

In a piecewise structural equation model, variables with no path specified between them are assumed to be independent after accounting for the paths which are specified. These "independence claims" were assessed using tests of directed separation. For linear mixed models, the degrees of freedom for these tests were calculated using the Kenward-Rogers approximation for the degrees of freedom in an F-test (Lefcheck, 2016). The independence claim between extra-group breeding and relatedness was fit using a GLMM and so to calculate the significance of the independence claim this model was fit using MCMCglmm (Hadfield, 2010). This model was fit as described in SM1 except that relatedness to top males was also included as a fixed effect. The results of these tests of directed separation are shown in Table S1. As all of the independence claims are non-significant we fail to reject them and we conclude that all meaningful relationships in our data are described by the paths specified in our piecewise structural equation model (see Table 2 and Table 3).

Table S1. Table of the independence claims and their results from the piecewise structural equation model presented in the main text. The independence claim is between the first two variables listed in each row conditional upon the subsequent variables in the row.

Independence claim	Estimate	Std.Error	DF	*p*-value
Brood f ~ group age + extra-group breeding*relatedness	0.01	0.05	24.21	0.89
Group size ~ in natal pack + group age	0.00	0.01	343.28	0.96
Brood f ~ in natal pack + extra-group breeding*relatedness	0.28	0.15	84.96	0.07
Group size ~ age + group age	0.00	0.00	169.25	0.48
Relatedness ~ age + group age + in natal pack	0.06	0.03	454.73	0.08
Brood f ~ age + extra-group breeding*relatedness	0.03	0.04	543.37	0.38
Relatedness ~ group size + group age + in natal pack	-0.02	0.04	273.17	0.56
Brood f ~ group size + group age + extra-group breeding*relatedness	0.05	0.04	251.45	0.18
Extra-group breeding ~ relatedness + group age + in natal pack + age + group size	0.30	0.18	NA	0.14

SM4 Animal model power analysis

In the main text we estimate individual repeatability in extra-group breeding behaviour by using individual identity as a random effect. In principle it is possible to separate individual variance into permanent environmental effects and additive genetic variance using an animal model (Kruuk & Hadfield, 2007; Wilson et al., 2010). However, animal models require large amounts of data and even then it is difficult to have an intuitive understanding of statistical power because it depends on several things such as the random effect structure and the distribution of relatedness across the pedigree (Wilson et al., 2010). Here we use simulations to show that we do not have sufficient explanatory power to resolve individual variance into its constituent permanent environmental variance and additive genetic variance.

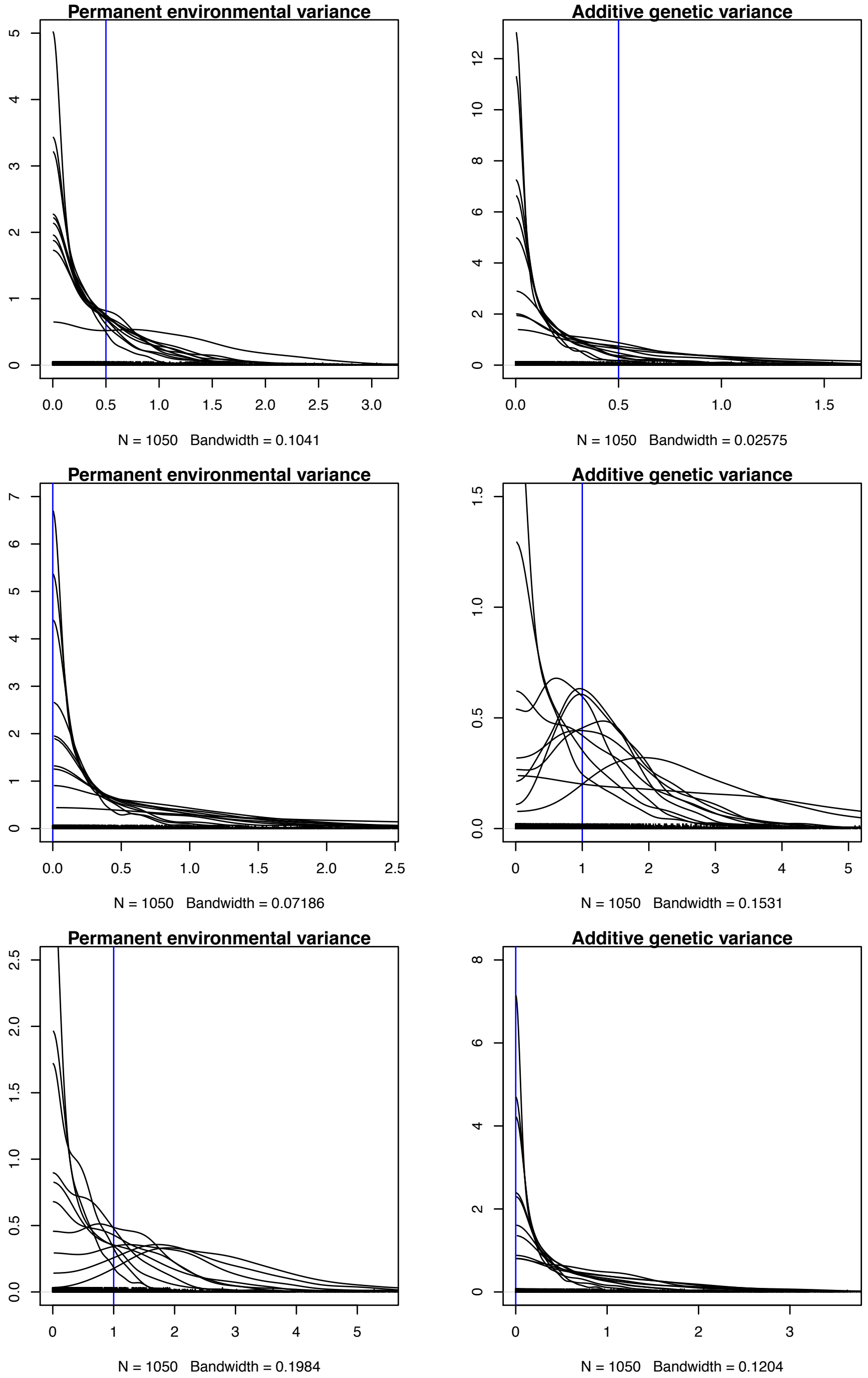
All of our simulations have the same random effect structure as our observed data, which is to say a brood was simulated with either with- or extra-group breeding status for each observed brood keeping the same mother, litter, and social group at conception. To simulate realistic data, the variance of each random effect was set to the posterior mean value estimated from the observed data. The individual level random effect was split between permanent environmental and additive genetic variance so that they summed to 1, the estimated individual variance. The variance in these terms was either split evenly (0.5, 0.5) or one term was assigned 0 variance and the other was variance of 1. Permanent environmental variance = 0, 1, or 0.5; additive genetic variance = 1, 0, or 0.5; social group at conception variance = 0.03; and litter variance = 5. The intercept for each level of the random effects was drawn from a normal distribution with mean 0 and the above specified variance except for the additive genetic variance which was simulated according to the observed pedigree using the phensim function in the R package pedantics (Morrissey, 2014). For a single brood, the probability of extra-group breeding on the link scale was the sum of the intercepts for the relevant mother (both permanent environmental and additive genetic intercepts), mother's group at conception, and litter. This link scale probability was converted to the data scale using the inverse logit link. Simulated broods were assigned extra-group status based on a single binomial trial where "success" was extra-group and "failure" was within-group and the probability of success was the above-mentioned data scale probability.

For each set of random effect variances, data was simulated 10 times and 10 animal models were fit. The random effect variances, including additive genetic variance, of the simulated data was estimated using GLMMs in the R package MCMCglmm (Hadfield, 2010). The only fixed effect was an intercept and the prior used the parameter expanded form for all random effects (V=1, nu = 0.002, alpha.V = 1000, alpha.mu = 0) except for the residual variance which was again fixed to 1 as it is inestimable when there is only a single realisation for each binomial event (Nakagawa & Schielzeth, 2010).

We also fit our original random effect structure from the main text (mother, litter, social group) to the simulated data where permanent environmental and additive genetic variance were equal. This original model estimated the individual variance and did not attempt to separate it into additive genetic and permanent environmental variances. This was in order to assess the power of our original model to accurately estimate individual level variance using our dataset.

Our simulations demonstrated little ability to accurately separate permanent environmental and additive genetic variance. When additive genetic and permanent environmental effects had equal non-zero variance (0.5) models were not able to accurately estimate either variance (Figure S4). The posterior distribution of both random effects had their modes at zero but uncertainty of the permanent environmental effect included one; the sum of the two variances. When all of the within-individual variance was assigned to permanent individual effects or additive genetic effect, models did not consistently resolve these effects accurately (Figure S4). The random effect that explained zero variance was generally estimated correctly. However, the mode of the posterior distribution of the random term which did explain variance was frequently zero, although the true value was within the posterior distribution (Figure S4). We also fit our original random effect structure, to simulated data with variance of 0.5 in both permanent environmental and additive genetic effects

(Figure S4). This confirmed that for our data structure, the individual variance in extra-group breeding could be estimated. However, all posterior distributions also overlapped zero and in some simulations the posterior mode was zero.



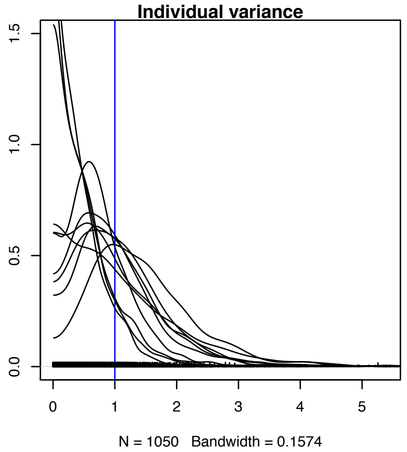


Figure S4. Density plots of the estimated random effect variances from models fit to simulated data. Each row of plots indicates a separate sets of simulation parameters, and these true parameters are indicated by the vertical blue lines. In the final row, data was simulated with a 0.5 mother specific variance and 0.5 additive genetic variance but the model did not attempt to separate them and so the individual variance is effectively 1.

In conclusion, despite a large pedigree and 662 separate broods, an animal model was unable to successfully estimate the permanent environmental variance and additive genetic variance; however, the individual variance was generally estimated correctly although the posterior distribution overlapped zero. As such in the main text we present only the individual level variance and not the results of the animal model. We also echo previous recommendations that simulations should be conducted for each dataset to determine the statistical power of the animal model (Morrissey, Wilson, Pemberton, & Ferguson, 2007; Wilson et al., 2010). To precisely assess statistical power will generally require many more simulations than we performed here but our simulations showed a clear inability to estimate separate genetic and environmental effects with relatively few simulations.

SM5 Group size and within-group breeding

We found that females in larger groups were less likely to breed with extra-group males. This could indicate that females in larger groups are able to avoid inbreeding despite within-group breeding because they have more potential within-group mates to choose from. In keeping with this idea, there is some evidence that banded mongooses preferentially breed with less related individuals when breeding within-group (Sanderson et al., 2015). If females in larger groups are more able to avoid within-group inbreeding, we would expect the inbreeding coefficient of broods fathered by within-group males to correlate with group size.

To test this hypothesis, we fit a linear mixed model to the data from broods sired by within-group males. The average brood inbreeding coefficient was the response variable while the fixed effects were the mother's related to top males and the group size. Similar to all other models we fit the mother, litter, and social group at conception as random effects. The significance of group size was assessed through parametric bootstrapping as described in the main text.

We found that group size did not significantly affect the average inbreeding coefficient of broods sired by within-group males (group size $ß = 0.08$, $p = 0.089$). Therefore, the observed lower probability of extra-group breeding in larger groups cannot be explained by females in larger groups successfully avoiding within-group inbreeding.

5

Thesis discussion

Inbreeding has been studied for well over a century, but there is currently a mismatch between theory, which predicts regular inbreeding under some circumstances, and empirical reports of inbreeding which are rare (Kokko & Ots, 2006; Szulkin, Stopher, Pemberton, & Reid, 2013). Darwin was the first to suggest that inbreeding depression had generated selection for species to avoid inbreeding (Darwin, 1876). This is a compelling narrative which has often been invoked to explain the evolution of many breeding behaviours (Pusey & Wolf, 1996). Inbreeding depression is well studied and has been reported across a wide range of taxa and as a consequence inbreeding avoidance is often expected to be the norm in the ecological literature (Szulkin et al., 2013). In opposition to this view, several recent papers have highlighted theoretical predictions that inbreeding should occur under some conditions because inbreeding depression will not inevitably select for inbreeding avoidance (Duthie, Lee, & Reid, 2016; Jamieson, Taylor, Tracy, Kokko, & Armstrong, 2009; Kokko & Ots, 2006; Lehtonen & Kokko, 2015; Puurtinen, 2011; Szulkin et al., 2013). Since this theory has gained more attention, inbreeding preference has been reported in cichlids, marmots, dolphins, and several bird species (Frère et al., 2010; Jacob, Prévot, & Baudry, 2016; Kleven, Jacobsen, Robertson, & Lifjeld, 2005; Olson, Blumstein, Pollinger, & Wayne, 2012; Thünken, Bakker, Baldauf, & Kullmann, 2007a; Townsend et al., 2018; Wang & Lu, 2011).

Inbreeding is clearly not always avoided as selfing, the most extreme form of inbreeding, is common among plants (Wright, Slotte, & Kalisz, 2013). An extensive body of literature has examined the factors governing the evolution of selfing in plants. However, several factors add complexity to biparental inbreeding and prevent this theory of selfing being applied to inbreeding more generally (Duthie & Reid, 2016; Szulkin et al., 2013). For instance, while selfing or outbreeding is a dichotomous decision, biparental inbreeding is a sliding scale which can occur between two individuals of any relatedness. Furthermore, the optimal degree of inbreeding is commonly expected to differ between the sexes and cause sexual conflict, something which is a non-issue in selfing (Kokko & Ots, 2006; Puurtinen, 2011; Waser, Peter, Austad, & Keane, 1986).

To help improve our understanding of biparental inbreeding, I have studied the causes and consequences of inbreeding in the banded mongoose. The banded mongoose is known to inbreed frequently, sometimes between close relatives, which is in contrast to the majority of cooperative breeders (Nichols, 2017) including the closely related meerkat (*Suricata suricatta*) where close inbreeding is never observed due to dispersal and not breeding with individuals known from early life (Nielsen et al., 2012). Another closely related species, the naked mole rat (*Heterocephalus glaber*), was once believed to be highly inbred; however, this conclusion appears to stem from sampling a small founder population and wider sampling found no such evidence for frequent inbreeding (Ingram, Troendle, Gill, Braude, & Honeycutt, 2015; reviewed in Nichols, 2017).

The high level of inbreeding in the banded mongoose is unlikely to be the result of population bottlenecks or reduced population size as in some other highly inbred vertebrates where generations of inbreeding result in inbreeding coefficients greater than those resulting from selfing (e.g. Kennedy *et al.*, 2014; Funk *et al.*, 2016). Instead, the high level of inbreeding observed in the banded mongoose is a consequence of their social group structure and high levels of natal philopatry in both sexes (Nichols, Cant, Hoffman, & Sanderson, 2014). As this unusual level of inbreeding is a natural occurrence, the banded mongoose can help us to understand the evolution of inbreeding in wild populations. In this discussion I identify four main factors which should determine the strength and direction of selection on inbreeding: inbreeding depression, kin selected benefits of inbreeding, the costs of inbreeding avoidance, and the risk of inbreeding. Below I consider the likely importance of each for the evolution of inbreeding, drawing both from the general literature and from my findings in the banded mongoose.

Inbreeding depression

The simplest explanation for frequent inbreeding in the banded mongoose would be the absence of inbreeding depression. When homozygous, the deleterious recessive alleles that are largely believed to be responsible for inbreeding depression (Charlesworth & Willis, 2009) are exposed to selection, and may consequently be reduced in frequency. As a result, the inbreeding load may decrease over several generations of inbreeding in a process known as purging (Crnokrak & Barrett, 2002; Hedrick & Garcia-Dorado, 2016; Leberg & Firmin, 2007). However, the extent of purging is inconsistent and difficult to predict (Boakes, Wang, & Amos, 2007; Caballero, Bravo, & Wang, 2017; Crnokrak & Barrett, 2002) and although purging is sometimes invoked to explain a lack of inbreeding depression (Laws & Jamieson, 2011) it is unlikely to be common in wild populations (Boakes et al., 2007; Keller & Waller, 2002) and is often absent despite demographic histories that should encourage purging (Jamieson, Wallis, & Briskie, 2006; Kennedy et al., 2014). It is therefore difficult to say when and how effectively purging should take place. In Chapter 2 I confirmed that despite frequent incest, presumably over many generations, inbreeding depression still markedly reduces fitness in the banded mongooses. This suggests that purging has not led to a low inbreeding load, and that a low inbreeding load is evidently not required for frequent inbreeding to evolve.

The genetic consequences of inbreeding may increase the likelihood of population and species extinction. Inbreeding is a major conservation concern, as it reduces individual fitness, which can contribute to population extinction (Frankham, 2005; O'Grady et al., 2006). Inbreeding also has more insidious genetic consequences; it reduces standing genetic diversity and therefore a population's ability to adapt to environmental changes (Frankham, 2003; Harrisson, Pavlova, Telonis-Scott, & Sunnucks, 2014) including diseases (Spielman, Brook, Briscoe, & Frankham, 2004). Because of these negative effects, Avilés & Purcell (2012) suggested that regular inbreeding may be an "evolutionary dead-end". An evolutionary dead-end is a trait which increase the probability of extinction and that, once the trait has evolved, is very unlikely to revert to the ancestral trait. There is quantitative support for this hypothesis in the Theridiidae family of social spiders where inbred societies are phylogenetically isolated, principally occurring on terminal branches despite eight or nine independent origins (Agnarsson, Avilés, Coddington, & Maddison, 2006). If inbreeding does represent an evolutionary dead-end, it could explain the apparent lack of regular inbreeding in the ecological literature. However, many of the examples considered in Avilés & Purcell (2012) are extreme cases of inbreeding and provide only mixed support for the evolutionary dead-end hypothesis. It seems unlikely then that milder forms of inbreeding should lead to extinction rapidly enough to explain their scarcity entirely.

The severity of inbreeding depression is not an unchangeable quantity but in general can be modified by stress. In chapter 3, I showed that offspring care, which should mitigate environmental stress, reduces inbreeding depression in offspring. Specifically, it reduced inbreeding depression in juvenile survival so much that it went undetected in chapter 2 (Wells *et al.*, 2018). This protective effect of care could reduce selection for inbreeding avoidance in species with high levels of offspring care. Indeed, Avilés & Bukowski (2006) suggest that such a protective effect could explain the repeated evolution of inbreeding and sociality in spiders. However, this protective effect is unlikely to completely explain frequent inbreeding in the banded mongoose, as care did not obscure inbreeding depression for male breeding success (chapter 2, Wells *et al.*, 2018). In fact the banded mongoose results demonstrate that frequent incest can persist despite continuing strong selection imposed by inbreeding depression.

The relationship between inbreeding and care may also influence the evolution of parental investment. When the level of population inbreeding increases, for example due to reduced effective population size, it could select for increased offspring care to counteract the increase in inbreeding. Such an effect may occur even though offspring care is unlikely to have originated to mitigate inbreeding depression (Pilakouta, Jamieson, Moorad, & Smiseth, 2015). This proposed increase in care is mirrored in the model of Duthie et al. (2016) which predicts that mothers should invest more resources into inbred offspring. Although my results do not support their prediction (Chapter 3) they do not necessarily contradict it either (see below), and my results show that care can mitigate inbreeding depression, which is an assumption of the model (Duthie et al., 2016).

Kin selection and inbreeding

Inbreeding may be selected for because of theoretical inclusive fitness benefits inherent to breeding with relatives (Lehtonen & Kokko, 2015; Parker, 1979; Waser, Peter et al., 1986). Parents are more related to inbred offspring and so inbred offspring possess more IBD alleles than equivalent outbred offspring. Therefore, inbreeding may increase fitness more than outbreeding if the benefits of increased genetic transmission outweigh the costs of inbreeding depression. This transmission advantage can allow alleles that promote inbreeding to increase in frequency and is analogous to the transmission advantage of an allele promoting selfing (Charlesworth, 2006; Charlesworth & Willis, 2009; Fisher, 1941).

This transmission advantage of inbreeding can alternatively be viewed as helping relatives to breed (Smith, 1979). Breeding with a relative may provide them with an additional breeding opportunity and this increased reproduction of a relative can be beneficial despite inbreeding depression in the offspring. The benefits of this “altruistic inbreeding” are strongly dependant on the specifics of the breeding system. Inbreeding will not be selected for if breeding with a relative prevents them from obtaining an unrelated mate (Lehtonen & Kokko, 2015). Viewing the transmission advantage of inbreeding as altruistic inbreeding makes it clear that the inclusive fitness of breeding with a relative depends on the fitness of that relative (Duthie & Reid, 2015).

Several theoretical studies over the last few years have incorporated this kin selected benefit of inbreeding to show that inbreeding can be selected for despite inbreeding depression (Duthie et al., 2016; Duthie & Reid, 2016; Kokko & Ots, 2006; Lehtonen & Kokko, 2015; Puurtinen, 2011). These studies have produced a range of interesting predictions including sex-specific levels of inbreeding preference (Kokko & Ots, 2006; Puurtinen, 2011), greater parental investment in inbred offspring (Duthie et al., 2016), and a strong effect of breeding ecology on inbreeding (Kokko & Ots, 2006; Lehtonen & Kokko, 2015).

In contrast to theoretical work, very few empirical studies have accounted for the transmission advantage of inbreeding when estimating reproductive success. Reid et al. (2015) advocate calculating the allelic value of offspring to a focal parent. This allelic value depends on the focal parent's inbreeding coefficient as well as their relatedness to their breeding partner and so this allelic value accounts for the transmission advantage of inbreeding. In populations where inbreeding is on average low, accounting for the allelic value of offspring seems to have little impact on estimates of fitness or selection (Reid et al., 2015; Troianou, Huisman, Pemberton, & Walling, 2018). This result is supported by Duthie & Reid (2016) where simulations show that the transmission advantage of inbreeding was only sufficient to increase the frequency of inbreeding preference alleles under a restricted set of conditions. The limited empirical evidence currently available suggests that this transmission advantage will be less important than inbreeding depression and the cost of inbreeding avoidance in determining the evolution of inbreeding behaviour in general. However, species which preferentially extra-pair breed with relatives may be important counter examples such as ground tits and barn swallows (Kleven et al., 2005; Wang & Lu, 2011).

Where inbreeding does occur, it may select for greater altruism because of its impact on relatedness. Theoretical work shows that parents with inbred offspring should invest more per offspring even when they are under selection to outbreed (Duthie et al., 2016). There are not many empirical tests of this prediction, but there is some support (Margulis, 1997; Thünken, Bakker, Baldauf, & Kullmann, 2007b; Thünken et al., 2007a) and cases of inbred individuals providing additional care (Nielsen, 2012; Sitkov-Sharon, Tremmel, Bouskila, Lubin, & Harari, 2017). In contrast, I found no evidence that inbred pups receive more care than outbred pups in the banded mongoose. This null result may occur because inbred pups cannot be identified amongst the mixed litter and so carers cannot preferentially care for inbred offspring.

Additional tests in other species are needed before firm conclusions can be drawn.

The costs of inbreeding avoidance

The cost of inbreeding avoidance behaviours could be an important determinant of the frequency of inbreeding across species. The effects of these costs have been studied in simulations and theoretical works which find that they limit the evolution of choosey behaviour and lead to inbreeding tolerance (Duthie & Reid, 2016; Jamieson et al., 2009). It can be difficult to measure the costs of inbreeding avoidance strategies, although many potential costs have been proposed, especially for extra-pair breeding (Table 1 in Forstmeier *et al.*, 2014). When females engage in extra-pair mating, the cuckolded male is expected to provide less offspring care; this reduction is among the best studied costs of inbreeding avoidance (reviewed in Arnqvist & Kirkpatrick, 2005). The fitness cost of inbreeding avoidance is likely to vary depending on the type of behaviour and, if these costs are generally important in determining the frequency of inbreeding, we may predict that species with more costly inbreeding avoidance strategies would display greater inbreeding tolerance. In keeping with this hypothesis, many of the invertebrate species with inbred social systems reviewed in Avilés & Purcell (2012) may have a high cost to inbreeding avoidance because the risk of failing to find a mate or breeding patch is ostensibly high as habitat patches are rare and short-lived or the distribution of mates is sparse and unpredictable. However, these costs have not yet been estimated empirically in these species. This prediction is also supported by results from the banded mongoose, where the costs of violence during extra-group breeding opportunities appear to select for within-group breeding and frequent incest (Chapter 4; Nichols, Cant and Sanderson, 2015).

In the banded mongoose, the cost of inbreeding avoidance is high and shapes breeding behaviour to the detriment of the entire population's genetic health. Banded mongoose groups compete violently over resources for short-term benefits (Thompson, Marshall, Vitikainen, & Cant, 2017). It seems that in order to avoid these violent inter-group interactions, females commonly breed with within-group males despite the risk of inbreeding (Chapter 4; Nichols, Cant and Sanderson, 2015). In which case, this violence ultimately causes inbreeding and low genetic fitness for both winners and losers of inter-group conflict.

The risk of inbreeding

The risk of inbreeding, here defined as the rate at which close relatives are encountered as potential mates, will intuitively control the potential selection on inbreeding. There is little to be gained by inbreeding avoidance if random mating rarely results in incestuous matings. Inbreeding avoidance or preference would have to be able to change the expected inbreeding coefficient of offspring in order to provide any fitness benefit. It follows then that selection for inbreeding avoidance or preference requires a mixture of relatives and non-relatives in the pool of potential mates. Jamieson et al. (2009) argue that New Zealand robins and saddlebacks are not selected to avoid inbreeding because the risk of inbreeding when mating randomly is so low.

Some breeding ecologies are associated with a high risk of inbreeding. For example, delayed dispersal and short dispersal distances are common in cooperative breeders, which means there is often the potential for inbreeding (Nichols, 2017). We may expect inbreeding avoidance to be more common in cooperative breeders than species with a lower risk of inbreeding because the risk of inbreeding is necessary to generate selection for inbreeding avoidance.

Indeed, a literature review in birds found that inbreeding avoidance through kin recognition had been reported in several cooperative species but only one pair breeding species (Jamieson et al., 2009). Although this trend is clear when they restrict their review to studies using pedigree data, the evidence is more equivocal when considering studies based on genetic data. A more recent meta-analysis using only genetic studies found a non-significant trend for cooperative species to show more evidence of inbreeding avoidance through extra-pair paternity (Arct, Drobniak, & Cichoń, 2015). These studies provide some evidence that the risk of inbreeding influences the evolution of inbreeding across species, but studies measuring the risk of inbreeding more precisely are needed for a more definitive answer.

If the risk of inbreeding is not fixed, we can study how it influences breeding behaviour within species. Individuals may plastically vary their behaviour to avoid (or seek out) inbreeding only when these behaviours are beneficial. In keeping with this, female Thomas langurs which have not bred only disperse if their father is present (van Hooff, Willems, Wich, & Sterck, 2005) and female marmots and badgers are more likely to avoid inbreeding through extra-pair or extra-group breeding when they are more related to within-pair or group males (Annavi et al., 2014; Cohas, Yoccoz, Da Silva, Goossens, & Allainé, 2006). Similarly, in chapter 4 I found that females are more likely to conceive to extra-group males when relatedness to within-group males is likely to be high. The ability of several species to adaptively vary their breeding behaviour with the risk of inbreeding indicates that this risk can be an important component of selection.

Future directions

In the banded mongoose the costs of intergroup interactions appear to drive the frequent incest we observed (Nichols et al., 2014, 2015; Wells et al., 2018). Unfortunately, it is difficult to collect data on intergroup interactions because observing them is a rare chance event; however, over the decades of study many have been observed (Thompson et al., 2017). Recently, one individual in each social group in the study population has been fitted with a Global Positioning System (GPS) collar. The data from these GPS collars should enable us to detect every intergroup interaction. In turn, this should allow us to more accurately estimate the cost of intergroup interactions and how it changes with individual and group conditions. Comparing the costs of intergroup interactions with estimates of inbreeding depression will help to objectively assess if these costs of inbreeding avoidance are large enough to select for the observed level of close inbreeding.

Many studies have inferred selection against inbreeding from inbreeding depression. Reid et al. (2015) point out however that this is selection against “being inbred” and that to directly estimate selection on inbreeding we must compare the fitness of individuals with related and unrelated breeding partners. So far I believe that this approach has only been used in Mandarte island song sparrows, red deer and the banner-tailed kangaroo rat (Reid et al., 2015; Troianou et al., 2018; Willoughby, Waser, Brüniche-Olsen, & Christie, 2019). It is in principal possible to decompose the overall selection pressure into cost of inbreeding depression, cost of inbreeding avoidance, and the kin selected benefits of inbreeding (Reid et al., 2015). Such decomposition will not only help to explain observed breeding patterns but also help to refine theoretical models with real world parameters. If the parameter combinations conducive to inbreeding preference are rare in nature it could explain the apparent lack of inbreeding preference in the wild.

To apply this approach, studies will need several generations of life history data and accurately assigned parentage. Collecting this data is both expensive and time consuming (Pemberton, 2008). Fortunately, there are already several long-term studies of natural populations where this approach could be employed. In particular this technique may help us to understand the evolution of inbreeding preference if applied to species which seek out related mates for example barn swallows, the cichlid *Pelvicachromis taeniatus*, and ground tits (Kleven *et al.*, 2005; Thünken *et al.*, 2007a; Wang and Lu, 2011) by estimating the relative importance of ecological constraints and kin selected benefits.

This approach could be applied to the banded mongoose as we have data on both inbreeding and breeding success over several generations. However, the impact of behaviours on related group members would also have to be considered as the banded mongoose is a cooperative breeder. Inter-group interactions involve the entire social group and so the costs of inbreeding avoidance will be paid by all; however, these costs may not be evenly spread across individuals. Furthermore, it will be difficult to estimate inbreeding depression in evolutionary fitness as inbred individuals also influence the fitness of others by providing less cooperative care (chapter 3). This approach clearly requires careful consideration before it can be applied to social species.

Conclusions

Inbreeding depression is the most well studied facet of inbreeding and is largely responsible for a focus on inbreeding avoidance in the ecological literature (Szulkin et al., 2013). However, this thesis highlights that, under certain circumstances, frequent inbreeding can become the norm despite severe inbreeding depression. Much theoretical work focuses on the kin selected benefits of inbreeding depression (Duthie et al., 2016; Duthie & Reid, 2016; Kokko & Ots, 2006; Lehtonen & Kokko, 2015; Puurtinen, 2011), but there is little empirical support that it plays a general role in the evolution of inbreeding. The two studies which have estimated how kin selection can change the selection pressure to inbreed (Reid et al., 2015; Troianou et al., 2018) suggest that kin selection benefits may be dwarfed by the costs of inbreeding avoidance and inbreeding depression. However, this may be fertile ground for future empirical work, especially in social species or those displaying inbreeding preference where kin selected benefits may be more important. Inbreeding is preferred in the cichlid *Pelviachromis taeniatus* where it reduces conflict between parents over biparental offspring care and offspring therefore receive more care (Thünken et al., 2007a). Inbreeding may also have a relatively low cost in this species due to purging (Langen, Schwarzer, Kullmann, Bakker, & Thünken, 2011). Finally, the costs of inbreeding avoidance behaviour appear to have been central to the evolution of inbreeding in the banded mongoose and consideration of other inbred species implies that they may be important in general.

Simple hypotheses that species do or do not avoid inbreeding may not sufficiently represent the complexity of natural systems. In chapter 4, I showed that females vary their inbreeding behaviour with their social environment. Perhaps if we ask how inbreeding behaviour changes with environmental conditions, we shall find more subtle effects with the same populations showing inbreeding avoidance, tolerance and preference as the risk of inbreeding and cost of inbreeding avoidance change. Identifying which variables are used by individuals to adjust their breeding behaviour will also help to identify factors that may be important across species in shaping the evolution of inbreeding behaviour.

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